Gene Expression in Breast Cancer

This application claims priority of U.S. Provisional Application No. 60/456,735, filed March 20, 2003, the disclosure of which is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

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TECHNICAL FIELD

This invention relates to breast cancer, and more particularly to genes expressed in breast cancer cells.

BACKGROUND

Ductal carcinoma *in situ* (DCIS) of the breast includes a heterogeneous group of pre-invasive breast tumors with a wide range of invasive potential. In order to initiate early aggressive treatment where needed but to avoid such treatment, and its frequent harsh side effects, where not needed, it is important that methods to distinguish between DCIS and invasive breast cancer and between different types of DCIS be developed.

SUMMARY

The invention is based on the inventors' discovery of differing patterns of gene expression in breast cancer cells versus normal cells, in DCIS cells versus invasive and/or metastatic breast cancer cells, and between different grades of DCIS. The invention thus includes methods of diagnosis, methods of treatment, nucleic acids corresponding to newly identified genes, polypeptides encoded by such genes, and methods of screening for gene expression.

More specifically, the invention features a method of diagnosis. The method includes the steps of: (a) providing a test sample of breast tissue; (b) determining the level of expression in

the test sample of a gene selected from those listed in Table 1; and (c) if the gene is expressed in the test sample at a lower level than in a control normal breast tissue sample, diagnosing the test sample as containing cancer cells.

The invention also provides a method of determining the grade of a ductal carcinoma in situ (DCIS). The method includes the steps of: (a) providing a test sample of DCIS tissue; (b) deriving a test expression profile for the test sample by determining the level of expression in the test sample of ten or more genes selected from those listed in Tables 2-16; (c) comparing the test expression profile to control expression profiles of the ten or more genes in control samples of high grade, intermediate grade, and low grade DCIS; (d) selecting the control expression profile that most closely resembles the test expression profile; and (e) assigning to the test sample a grade that matches the grade of the control expression profile selected in step (d). The ten or more genes can be: 25 or more genes; 50 or more genes; 100 or more genes; 200 or more genes; 500 or more genes.

Another aspect of the invention is a method of determining the likelihood of a breast cancer being DCIS or invasive breast cancer. The method includes the steps of: (a) providing a test sample of breast tissue; (b) determining the level of expression in the test sample of a gene selected from the group consisting of a gene encoding CD74, a gene encoding MGC2328, a gene encoding S100A7, a gene encoding KRT19, a gene encoding trefoil factor 3 (TFF3), a gene encoding osteonectin, and a gene identified by a SAGE tag consisting of the nucleotide sequence CTGGGCGCCC; and (c) determining whether the level of expression of the selected gene in the test sample more closely resembles the level of expression of the selected gene in control cells of (i) DCIS or (ii) invasive breast cancer; and (d) classifying the test sample as: (i) likely to be DCIS if the level of expression of the gene in the test sample more closely resembles the level of expression of the gene in DCIS cells; or (ii) likely to be invasive breast cancer if the level of expression of the gene in the test sample more closely resembles the level of expression of the gene in invasive breast cancer cells.

Also embraced by the invention is a method of predicting the prognosis of a breast cancer patient. The method includes the steps of: (a) providing a sample of primary invasive breast cancer tissue from a test patient; and (b) determining the level of expression in the sample of a gene encoding S100A7 or a gene encoding fatty acid synthase (FASN). A level of expression

higher than in a control sample of primary invasive breast carcinoma from a patient with a good prognosis is an indication that the prognosis of the test patient is poor.

Another method of diagnosis includes the steps of: (a) providing a test sample of breast tissue comprising a test stromal cell; and (b) determining the level of expression in the stromal cell of a gene selected from those listed in Tables 7, 8 and 10, 15, and 16, the gene being one that is expressed in a cell of the same type as the test stromal cell at a substantially higher level when present in breast cancer tissue than when present in normal breast tissue; and (c) classifying the test sample as: (i) normal breast tissue if the level of expression of the gene in the test stromal cell is not substantially higher than a control level of expression for a cell of the same type as the test stromal cell in normal breast tissue; (ii) breast cancer tissue if the level of expression of the gene in the test stromal cell is substantially higher than a control level of expression for a cell of the same type as the test stromal cell in normal breast tissue. The stromal cells in the test sample and the standard samples can be leukocytes and the genes selected from those listed in Tables 7 and 15, e.g., genes encoding, for example, interleukin-1β (IL1β) or macrophage inhibitory protein 1α (MIP1α). The stromal cells in the test sample and the standard samples can also be myoepithelial cells or myofibroblasts and the genes selected from those listed in Tables 8, 15, and 16, e.g., genes encoding cathepsins F, K, and L, MMP2, PRSS11, thrombospondin 2, SERPING1, cytostatin C, TIMP3, platelet-derived growth factor receptor β-like (PDGFRBL), a collagen, collagen triple helix repeat containing 1 (CTHRC1), CXCL12, or CXCL14. The stromal cells in the test sample and the standard samples can be endothelial cells and the genes selected from those listed in Tables 10 and 15. Moreover, the stromal cells in the test sample and the standard samples can be fibroblasts and the genes selected from those listed in Table 15.

Another feature of the invention is method of diagnosis that involves: (a) providing a test sample of breast tissue comprising a test stromal cell; and (b) determining the level of expression in the stromal cell of a gene selected from those listed in Tables 7, 8, 10, and 15, the gene being one that is expressed in a cell of the same type as the test stromal cell at a substantially higher level when present in normal breast tissue than when present in breast cancer tissue; and (c) classifying the test sample as: (i) normal breast tissue if the level of expression of the gene in the test stromal cell is not substantially lower than a control level of expression for a cell of the same type as the test stromal cell in normal breast tissue; (ii) breast cancer tissue if the level of expression of the gene in the test stromal cell is substantially lower than a control level of

expression for a cell of the same type as the test stromal cell in normal breast tissue. The stromal cells in the test sample and the standard samples can be leukocytes and the genes selected from those listed in Tables 7 and 15. Alternatively, the stromal cells in the test sample and the standard samples can be myoepithelial cells or myofibroblasts and the genes selected from those listed in Tables 8 and 15. Furthermore, the stromal cells in the test sample and the standard samples can be endothelial cells and the genes can be selected from those listed in Tables 10 and 15. In addition, the stromal cells in the test sample and the standard samples can be fibroblasts and the genes selected from those listed in Table 15.

In another aspect, the invention provides a method of diagnosis that involves: (a) providing a test sample of breast tissue comprising a test epithelial cell of the luminal epithelial type; (b) determining the level of expression in the test epithelial cell of a gene selected from those listed in Tables 9 and 15, the gene being one that is expressed in cancerous epithelial cells of the luminal epithelial cell type at a substantially higher level than those in normal breast tissue; and (c) classifying the test sample as: (i) normal breast tissue if the level of expression of the gene in the test epithelial cell is not substantially higher than a control level of expression for an epithelial cell of luminal epithelial cell type in normal breast tissue; (ii) breast cancer tissue if the level of expression of the gene in the test epithelial cell is substantially higher than a control level of expression for an epithelial cell of the luminal epithelial type in normal breast tissue.

Also featured by the invention is a method of diagnosis that includes: (a) providing a test sample of breast tissue comprising a test epithelial cell of the luminal epithelial type; and (b) determining the level of expression in the test epithelial cell of a gene selected from those listed in Table 9, the gene being one that is expressed in epithelial cells of the luminal epithelial cell type at a substantially lower level when present in breast cancer tissue than when present in normal breast tissue; and (c) classifying the test sample as: (i) normal breast tissue if the level of expression of the gene in the test epithelial cell is not substantially lower than a control level of expression for an epithelial cell of luminal epithelial cell type in normal breast tissue; (ii) breast cancer tissue if the level of expression of the gene in the test epithelial cell is substantially lower than a control level of expression for an epithelial cell of the luminal epithelial type in normal breast tissue.

In all the above methods of the invention the level of expression of the gene can determined as a function of the level of protein encoded by the gene or as a function of the level of mRNA transcribed from the gene.

Another embodiment of the invention is a method of inhibiting proliferation or survival of a breast cancer cell. The method involves contacting a breast cancer cell with a polypeptide that is encoded by a gene selected from those listed in Tables 1, 7-10, and 15, the gene being one that is expressed in the cancer cell, or a stromal cell in a tumor comprising the cancer cell, at a level substantially lower than in a normal cell of the same type. In the method, the cancer cell can be *in vitro*. Alternatively, it can be in a mammal, e.g., a human. The contacting can include administering the polypeptide to the mammal or administering a polynucleotide encoding the polypeptide to the mammal. The method can also involve: (a) providing a recombinant cell that is the progeny of a cell obtained from the mammal and has been transfected or transformed *ex vivo* with a nucleic acid encoding the polypeptide; and (b) administering the recombinant cell to the mammal, so that the recombinant cell expresses the polypeptide in the mammal.

Another feature of the invention is a method of inhibiting pathogenesis of a breast cancer cell or stromal cell in a tumor of a mammal. The method includes: (a) identifying a mammal with a breast cancer tumor; and (b) administering to the mammal an agent that inhibits binding of a polypeptide encoded by a gene selected from those listed in Tables 2-10, 15, and 16 to its receptor or ligand, the gene being one that is expressed in a breast cancer cell in the tumor, or in a stromal cell in the tumor, at a level substantially higher than in a corresponding cell in a non-cancerous breast. The polypeptide is a secreted polypeptide or a cell-surface polypeptide. The agent can be a non-agonist antibody that binds to the polypeptide, a soluble form of the receptor, or a non-agonist antibody that binds to the receptor or ligand. The polypeptide can be, for example, CXCL12 or CXCL14 and the receptor can be, for example, CXCR4 or a receptor for CXCL14.

Another aspect of the invention is a method of inhibiting expression of a gene in a cell. The method includes introducing into a target cell selected from the group consisting of (a) a breast cancer cell and (b) stromal cell in a tumor comprising a breast cancer cell, an agent that inhibits expression of a gene selected from those listed in Tables 2-10, 15, and 16, the gene being one that is expressed in the target cell at a level substantially higher than in a corresponding cell in normal breast tissue. The agent can be an antisense oligonucleotide that

hybridizes to an mRNA transcribed from the gene. The introducing step can involve administration of the antisense oligonucleotide to the target cell. The introducing step comprises administering to the target cell a nucleic acid comprising a transcriptional regulatory element (TRE) operably linked to a nucleotide sequence complementary to the antisense oligonucleotide, wherein transcription of the nucleotide sequence inside the target cell produces the antisense oligonucleotide. The agent can also be an RNAi molecule, one strand of the RNAi molecule having the ability to hybridize to a mRNA transcribed from the gene. The agent can also be a small molecule that inhibits expression of the gene. The gene can be one that encodes, for example, can be, for example, CXCL12, CXCL14 , CXCR4, or a receptor for CXCL14.

Also provided by the invention is an isolated DNA that includes: (a) the nucleotide sequence of a tag selected from those listed in Fig. 7; or (b) the complement of the nucleotide sequence. Also embraced by the invention is a vector containing the DNA. In the vector, the DNA can optionally be operatively linked to a transcriptional regulatory element (TRE). A cell comprising any of the vectors of the invention is also an aspect of the invention. Also included in the invention is an isolated polypeptide encoded by the DNA of the invention.

In another aspect, the invention embraces a single stranded nucleic acid probe that includes: (a) the nucleotide sequence of a tag selected from those listed in Tables 1-5, 7-10, 15, and 16; or (b) the complement of the nucleotide sequence.

Also embodied by the invention is an array that includes a substrate having at least 10 addresses, each address having disposed on it a capture probe that includes a nucleic acid sequence consisting of a tag nucleotide sequence selected from those listed in Tables 1-5, 7-10, 15, and 16. The tag nucleotide sequence can be one that corresponds to a gene encoding a protein selected from the group consisting of fatty acid synthase (FASN), trefoil factor 3 (TFF3), X-box binding protein 1 (XBP1), interferon alpha inducible protein 6-16 (IFI-6-16), cysteine-rich protein 1 (CRIP1), interferon-stimulated protein 15 kDa (ISG15), interferon alpha inducible protein 27 (IFI27), brain expressed X linked 1 (BEX1), helicase/primase protein (LOC150678), anaphase promoting complex subunit 11 (ANAPC11), Fer-1-like 4 (FER1L4), psoriasin, connective tissue growth factor (CTGF), regulator of G-protein signaling 5 (RGS5), paternally expressed 10 (PEG10), osteonectin (SPARC), LOC51235, CD74, MGC23280, Invasive Breast Cancer 1 (IBC-1), Apolipoprotein D (APOD), carboxypeptidase B1 (CPB1), retinal binding protein 1 (RBP1), FLJ30428, calmodulin-like skin protein (CLSP), nudix (NUDT8),

MGC14480, interleukin-1β (ILβ), macrophage inhibitory protein 1α (MIP1α), cathepsins F, K, and L, MMP2, PRSS11, thrombospondin 2, SERPING1, cytostatin C, TIMP3, platelet-derived growth factor receptor β-like (PDGFRBL), a collagen, collagen triple helix repeat containing 1 (CTHRC1), CXCL12, CXCL14, and a protein encoded by a gene identified by a SAGE tag consisting of the nucleotide sequence CTGGGCGCCC. The array can contain at least 25 addresses; at least 50 addresses; at least 100 addresses; at least 200 addresses; or at least 500 addresses.

The invention also features a kit comprising at least 10 probes, each probe including a nucleic acid sequence that includes a tag nucleotide sequence selected from those listed in Tables 1-5, 7-10, 15, and 16. The kit can contain at least 25 probes; at least 50 probes; at least 100 probes; at least 200 probes; at least 500 probes.

Another kit provided by the invention is one that contains at least 10 antibodies each of which is specific for a different protein encoded by a gene identified by a tag selected from the group consisting of the tags listed in Tables 1-5, 7-10, 15, and 16. The antibodies can, for example, be specific for a protein selected from the group consisting of fatty acid synthase (FASN), trefoil factor 3 (TFF3), X-box binding protein 1 (XBP1), interferon alpha inducible protein 6-16 (IF1-6-16), cysteine-rich protein 1 (CRIP1), interferon-stimulated protein15 kDa (ISG15), interferon alpha inducible protein 27 (IFI27), brain expressed X linked 1 (BEX1), helicase/primase protein (LOC150678), anaphase promoting complex subunit 11 (ANAPC11), Fer-1-like 4 (FER1L4), psoriasin, connective tissue growth factor (CTGF), regulator of G-protein signaling 5 (RGS5), paternally expressed 10 (PEG10), osteonectin (SPARC), LOC51235, CD74, MGC23280, Invasive Breast Cancer 1 (IBC-1), Apolipoprotein D (APOD), carboxypeptidase B1 (CPB1), retinal binding protein 1 (RBP1), FLJ30428, calmodulin-like skin protein (CLSP), nudix (NUDT8), MGC14480, interleukin-1β (ILβ), macrophage inhibitory protein 1α (MIP1α), cathepsins F, K, and L, MMP2, PRSS11, thrombospondin 2, SERPING1, cytostatin C, TIMP3, platelet-derived growth factor receptor β-like (PDGFRBL), a collagen, collagen triple helix repeat containing 1 (CTHRC1), CXCL12, CXCL14, and a protein encoded by a gene identified by a SAGE tag consisting of the nucleotide sequence CTGGGCGCCC. The kit can contain at least 25 antibodies; at least 50 antibodies; at least 100 antibodies; at least 200 antibodies; or at least 500 antibodies.

In addition the invention provides a method of identifying the grade of a DCIS. The method involves: (a) providing a test sample of DCIS tissue; (b) using the above-described array to determine a test expression profile of the sample; (c) providing a plurality of reference profiles, each derived from a DCIS of a defined grade, the test expression profile and each reference profile having a plurality of values, each value representing the expression level of a gene corresponding to a tag selected from those listed in Tables 1-5, 7-10, 15, and 16; and (d) selecting the reference profile most similar to the test expression profile, to thereby identify the grade of the test DCIS.

In another embodiment, the invention provides a method of determining whether a breast cancer is a DCIS or an invasive breast cancer. The method involves: (a) providing a test sample of breast cancer tissue; (b) determining the level of expression of CXCL14 in myofibroblasts in the test sample; (c) determining whether the level of expression of CXCL14 in the myofibroblasts in the test sample more closely resembles the level of expression of CXCL14 in control myofibroblasts of (i) DCIS or (ii) invasive breast cancer; and (d) classifying the test sample as: (i) DCIS if the level of expression of CXCL14 in myofibroblasts in the test sample more closely resembles the level of expression of CXCL14 in control myofibroblasts of DCIS; (ii) invasive breast cancer if the level of expression of CXCL14 in myofibroblasts in the test sample more closely resembles the level of expression of CXCL14 in control myofibroblasts of invasive breast cancer.

Polypeptide" and "protein" are used interchangeably and mean any peptide-linked chain of amino acids, regardless of length or post-translational modification.

The term "isolated" polypeptide or peptide fragment as used herein refers to a polypeptide or a peptide fragment which either has no naturally-occurring counterpart or has been separated or purified from components which naturally accompany it, e.g., in tissues such as pancreas, liver, spleen, ovary, testis, muscle, joint tissue, neural tissue, gastrointestinal tissue, or breast tissue or tumor tissue (e.g., breast cancer tissue), or body fluids such as blood, serum, or urine. Typically, the polypeptide or peptide fragment is considered "isolated" when it is at least 70%, by dry weight, free from the proteins and other naturally-occurring organic molecules with which it is naturally associated. Preferably, a preparation of a polypeptide (or peptide fragment thereof) of the invention is at least 80%, more preferably at least 90%, and most preferably at least 99%, by dry weight, the polypeptide (or the peptide fragment thereof),

respectively, of the invention. Since a polypeptide that is chemically synthesized is, by its nature, separated from the components that naturally accompany it, the synthetic polypeptide is "isolated."

An isolated polypeptide (or peptide fragment) of the invention can be obtained, for example, by extraction from a natural source (e.g., from tissues or bodily fluids); by expression of a recombinant nucleic acid encoding the polypeptide; or by chemical synthesis. A polypeptide that is produced in a cellular system different from the source from which it naturally originates is "isolated," because it will necessarily be free of components which naturally accompany it. The degree of isolation or purity can be measured by any appropriate method, e.g., column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

An "isolated DNA" is either (1) a DNA that contains sequence not identical to that of any naturally occurring sequence, or (2), in the context of a DNA with a naturally-occurring sequence (e.g., a cDNA or genomic DNA), a DNA free of at least one of the genes that flank the gene containing the DNA of interest in the genome of the organism in which the gene containing the DNA of interest naturally occurs. The term therefore includes a recombinant DNA incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote. The term also includes a separate molecule such as: a cDNA where the corresponding genomic DNA has introns and therefore a different sequence; a genomic fragment that lacks at least one of the flanking genes; a fragment of cDNA or genomic DNA produced by polymerase chain reaction (PCR) and that lacks at least one of the flanking genes; a restriction fragment that lacks at least one of the flanking genes; a DNA encoding a non-naturally occurring protein such as a fusion protein, mutein, or fragment of a given protein; and a nucleic acid which is a degenerate variant of a cDNA or a naturally occurring nucleic acid. In addition, it includes a recombinant nucleotide sequence that is part of a hybrid gene, i.e., a gene encoding a non-naturally occurring fusion protein. It will be apparent from the foregoing that isolated DNA does not mean a DNA present among hundreds to millions of other DNA molecules within, for example, cDNA or genomic DNA libraries or genomic DNA restriction digests in, for example, a restriction digest reaction mixture or an electrophoretic gel slice.

As used herein, a "functional fragment" of a polypeptide is a fragment of the polypeptide that is shorter than the full-length, mature polypeptide and has at least 5% (e.g., at least: 5%; 10%; 20%; 30%; 40%; 50%; 60%; 70%; 80%; 90%; 95%; 98%; 99%; 100%; or more) of the

activity (e.g., ability to inhibit proliferation of breast cancer cells) of the full-length, mature polypeptide. Fragments of interest can be made either by recombinant, synthetic, or proteolytic digestive methods. Such fragments can then be isolated and tested for their ability, for example, to inhibit the proliferation of cancer cells as measured by [^{3}H]-thymidine incorporation or cell counting.

As used herein, "operably linked" means incorporated into a genetic construct so that expression control sequences effectively control expression of a coding sequence of interest.

As used herein, the term "antibody" refers not only to whole antibody molecules, but also to antigen-binding fragments, e.g., Fab, F(ab')$_2$, Fv, and single chain Fv (ScFv) fragments. Also included are chimeric antibodies.

As used herein, the term "pathogenesis" of a cell (e.g., a cancer cell or stromal cell within a tumor containing a cancer cell) means proliferation of a cell, survival of a cell, invasiveness of a cell, migratory potential of a cell, metastatic potential of cell, ability of a cell to evade immune effector mechanisms, ability of a cell to induce or enhance angiogenesis, or ability of a cell to induce or enhance lymphangenesis.

As used herein, a gene that is expressed at a "substantially higher level" in a first cell (or first issue) than in a second cell (or second tissue) is a gene that is expressed in the first cell (or tissue) at a level at least 2 (e.g., at least: 2; 3; 4; 5; 6; 7; 8; 9; 10; 15; 20; 30; 40; 50; 75; 100; 200; 500; 1,000; 2000; 5,000; or 10,000) times higher than in the second cell (or second tissue).

As used herein, a gene that is expressed at a "substantially lower level" in a first cell (or first issue) than in a second cell (or second tissue) is a gene that is expressed in the first cell (or tissue) at a level at least 2 (e.g., at least: 2; 3; 4; 5; 6; 7; 8; 9; 10; 15; 20; 30; 40; 50; 75; 100; 200; 500; 1,000; 2000; 5,000; or 10,000) times lower than in the second cell (or second tissue).

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting.

Other features and advantages of the invention, e.g., diagnosing breast cancer, will be apparent from the following description, from the drawings and from the claims.

DESCRIPTION OF DRAWINGS

Fig. 1 is diagrammatic representation of the antibody-based procedure used to purify epithelial and stromal cells from DCIS and normal breast tissue for the analysis described in Example 6.

Fig. 2 is a series of photographs of ethidium bromide-stained electrophoretic gels of the products of RT-PCRs. The RT-PCR analysis was carried out on mRNA isolated from: (a) luminal epithelial cells ("epithelium"), myoepthelial cells ("myoepithelium"), leukocytes, and endothelial cells ("endothelium") purified from two DCIS tumor sample ("DCIS6" and "DCIS7"); and (b) leukocytes and endothelial cells ("endothelium") from normal breast tissue ("Normal"). The PCR phases of the RT-PCRs were carried out with oligonucleotide primers specific for two constitutively expressed genes (β-actin ("BAC") and L19) and for HER2 (expressed by some breast cancers), CALLA (a myoepithelial cell marker), CD45 (a pan-leukocyte marker), and a cell surface protein specifically expressed by endothelial cells ("CDH5"). The numbers at the bottom of each column of photographs ("25", "30", and "35") indicate numbers of PCR cycles.

Fig. 3A is a dendrogram showing the relatedness of SAGE libraries generated from normal mammary luminal epithelial cells (N1 and N2), DCIS cells (D1-D7 and T18), primary invasive breast cancer cells (I1-I6), breast cancer cells in lymph node metastases (LN1 and LN2), and breast cancer cells in a distant lung metastasis (M1) and analyzed by hierarchical clustering.

Fig. 3B is a dendrogram showing similarities among intermediate and high grade DCIS tumor SAGE libraries analyzed by hierarchical clustering using 582 genes.

Fig. 3C is a dendrogram showing similarities among intermediate and high grade DCIS tumor SAGE libraries analyzed by hierarchical clustering using 26 genes selected from the 582 genes used for the analysis depicted in Fig. 1B.

Fig. 4A is a series of photomicrographs showing the hybridization of riboprobes corresponding to genes encoding IFI-6-16, S100A7, CTGF, and RGS5 to frozen sections of DCIS tumors (T18, 96-331, 6164) and normal breast tissue (N24). Strong expression (indicated by dark staining) of IFI-6-16 and S100A7 is detected in tumor cells of a subset of DCIS tumors

but not in normal breast tissue epithelial cells. Expression of CTGF and RGS5 is seen mostly in DCIS stromal fibroblasts and myoepithelial cells, respectively, but not in the corresponding cells in normal breast tissue.

Fig. 4B is dendrogram showing the relatedness of five normal breast tissues, and 18 DCIS and invasive tumors analyzed for expression of 14 genes (SCGB3A1, TM4SF1, CTGF, XBP1, IFI27, ISG15, RGS5, RGS5, LOC150678, BEX1, PEG10, IFI-6-16, TFF3, CRIP1, S100A7, and CTGF) by mRNA *in situ* hybridization. Numbers are specimen identifiers. "N" denotes normal breast tissue, "D" denotes DCIS tissue, and "I" denotes invasive breast cancer tissue.

Fig. 4C is series of photomicrographs showing immunohistochemical staining of sections of a representative DCIS tumor in a tissue microarray. The tissue sections were stained with monoclonal antibodies specific for the indicated proteins. Dark staining indicates the presence of the protein. The data thus indicate the presence of S100A7, TFF3, SPARC, and CTGF but absence of IBC-1 in the DCIS tumor.

Fig. 5 is diagrammatic representation of the antibody-based procedure used to purify epithelial and stromal cells from DCIS and normal breast tissue for the analysis described in Example 7.

Fig. 6A is a line graph depicting the results of a Scatchard analysis of alkaline phosphate (AP) conjugated CXCL14 (AP-CXCL14) binding to MDA-MB-231 breast cancer cells.

Fig. 6B is a series of line graphs showing the effect of AP-CXCL14 (left and right panels) and CXCL12 (center panel) on the growth of MDA-MB-231 breast cancer cells (left and center panels) and MCF10A immortalized normal breast epithelial cells (right panel).

Fig. 6C is a pair of bar graphs showing the ability of CXCL14 N-terminally conjugated with AP (AP-CXCL14), or C-terminally conjugated with AP (CXCL14-AP), to enhance migration (left panel) and invasion (right panel) of MDA-MB-231 breast cancer cells. The cultures containing the CXCL14 conjugates (and corresponding control cultures) were in serum-free medium. Data from control cultures carried out in medium containing 10% FBS and no CXCL14 conjugate are shown ("10% FBS").

Fig. 7 is a depiction of the nucleotide sequences of SAGE tags that are listed in Tables 1-4, 7, 8, 10, and 15 and that correspond to no cDNA or mRNA nucleotide sequences present in the publicly available databases searched by the inventors.

DETAILED DESCRIPTION

Various aspects of the invention are described below.

Nucleic Acid Molecules

The nucleic acid molecules of the invention include those containing or consisting of the nucleotide sequences (or the complements thereof) of the SAGE (serial analysis of gene expression) tags listed in Fig. 7. The nucleic acid molecules of the invention can be cDNA, genomic DNA, synthetic DNA, or RNA, and can be double-stranded or single-stranded (i.e., either a sense or an antisense strand). Segments of these molecules are also considered within the scope of the invention, and can be produced by, for example, the polymerase chain reaction (PCR) or generated by treatment with one or more restriction endonucleases. A ribonucleic acid (RNA) molecule can be produced by *in vitro* transcription. Preferably, the nucleic acid molecules encode polypeptides that, regardless of length, are soluble under normal physiological conditions.

The nucleic acid molecules of the invention can contain naturally occurring sequences, or sequences that differ from those that occur naturally, but, due to the degeneracy of the genetic code, encode the same polypeptide. In addition, these nucleic acid molecules are not limited to coding sequences, e.g., they can include some or all of the non-coding sequences that lie upstream or downstream from a coding sequence. They can also contain irrelevant sequences at their 5' and/or 3' ends (e.g., sequences derived from a vector).

The nucleic acid molecules of the invention can be synthesized (for example, by phosphoramidite-based synthesis) or obtained from a biological cell, such as the cell of a mammal. The nucleic acids can be those of a human, non-human primate (e.g., monkey), mouse, rat, guinea pig, cow, sheep, horse, pig, rabbit, dog, or cat. Combinations or modifications of the nucleotides within these types of nucleic acids are also encompassed.

In addition, the isolated nucleic acid molecules of the invention encompass segments that are not found as such in the natural state. Thus, the invention encompasses recombinant nucleic acid molecules incorporated into a vector (for example, a plasmid or viral vector) or into the genome of a heterologous cell (or the genome of a homologous cell, at a position other than the natural chromosomal location). Recombinant nucleic acid molecules and uses therefor are discussed further below.

Techniques associated with detection or regulation of genes are well known to skilled artisans. Such techniques can be used to diagnose and/or treat disorders (e.g., DCIS or invasive cancer) associated with aberrant expression of the genes corresponding to the SAGE tags listed in Fig. 7.

Family members of the genes or proteins or proteins of the invention can be identified based on their similarity to the relevant gene or protein, respectively. For example, the identification can be based on sequence identity. The invention features isolated nucleic acid molecules which are at least 50% (or at least: 55%; 65%; 75%; 85%; 95%; 98%; 99%; 99.5%; or even 100%) identical to: (a) nucleic acid molecules that encode polypeptides encoded by genes corresponding to the SAGE tags listed in Fig. 7; (b) the nucleotide sequences of the coding regions of genes corresponding to the SAGE tags listed in Fig. 7; (c) nucleic acid molecules that include a segments of at least 30 (e.g., at least: 40; 50; 60; 80; 100; 125; 150; 175; 200; 250; 300; 500; 700;1,000; 2,000; 3000; 5,000, 10,000; or more) nucleotides of the coding regions of genes corresponding to the SAGE tags listed in Fig. 7; and (d) nucleic acid molecules that include the genomic sequences of genes corresponding to the SAGE tags listed in Fig. 7; (e) nucleic acid molecules that include a segments of at least 30 (e.g., at least: 40; 50; 60; 80; 100; 125; 150; 175; 200; 250; 300; 500; 700;1,000; 2,000; 3000; 5,000, 10,000; or more) nucleotides of the genomic sequences of genes listed corresponding to the SAGE tags listed in Fig. 7; (f) nucleic acid molecules containing or consisting of the SAGE tags listed in Fig. 7.

The determination of percent identity between two sequences is accomplished using the mathematical algorithm of Karlin and Altschul [(1990) Proc. Natl. Acad. Sci. USA 87:2264-2268] modified as in Karlin and Altschul [(1993) Proc. Natl. Acad. Sci. USA 90: 5873-5877]. Such an algorithm is incorporated into the BLASTN and BLASTP programs of Altschul et al. [(1990) J. Mol. Biol. 215: 403-410]. BLAST nucleotide searches are performed with the BLASTN program, score = 100, wordlength = 12, to obtain nucleotide sequences homologous to any of the nucleic acid molecules described herein. BLAST protein searches are performed with the BLASTP program, score = 50, wordlength = 3, to obtain amino acid sequences homologous to the polypeptides by encoded by any of the nucleic acid molecules described herein. To obtain gapped alignments for comparative purposes, Gapped BLAST is utilized as described in Altschul et al. [(1997) Nucleic Acids Res. 25:3389-3402]. When utilizing BLAST and Gapped BLAST

programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) are used.

Hybridization can also be used as a measure of homology between two nucleic acid sequences. A nucleic acid sequence, or a portion thereof, can be used as a hybridization probe according to standard hybridization techniques. The hybridization of a nucleic acid probe specific for a target DNA or RNA of interest to DNA or RNA from a test source (e.g., a mammalian cell) is an indication of the presence of the target DNA or RNA in the test source. Hybridization conditions are known to those skilled in the art and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y., 6.3.1-6.3.6, 1991. Moderate hybridization conditions are defined as equivalent to hybridization in 2 X sodium chloride/sodium citrate (SSC) at 30°C, followed by a wash in 1 X SSC, 0.1% SDS at 50°C. Highly stringent conditions are defined as equivalent to hybridization in 6 X sodium chloride/sodium citrate (SSC) at 45°C, followed by a wash in 0.2 X SSC, 0.1% SDS at 65°C.

The invention also encompasses: (a) vectors (see below) that contain any of the foregoing coding sequences and/or their complements (that is, "antisense" sequences); (b) expression vectors that contain any of the foregoing coding sequences operably linked to any transcriptional/translational regulatory elements (examples of which are given below) necessary to direct expression of the coding sequences; (c) expression vectors encoding, in addition to a polypeptide encoded by any of the foregoing sequences, a sequence unrelated to the polypeptide, such as a reporter, a marker, or a signal peptide fused to the polypeptide; and (d) genetically engineered host cells (see below) that contain any of the foregoing expression vectors and thereby express the nucleic acid molecules of the invention.

Recombinant nucleic acid molecules can contain a sequence encoding a polypeptide of the invention having a heterologous signal sequence. The full length polypeptide of the invention, or a fragment thereof, may be fused to such heterologous signal sequences or to additional polypeptides, as described below. Similarly, the nucleic acid molecules of the invention can encode the mature forms of the polypeptides of the invention or forms that include an exogenous polypeptide that facilitates secretion.

The transcriptional/translational regulatory elements referred to above include but are not limited to inducible and non-inducible promoters, enhancers, operators and other elements that are known to those skilled in the art and that drive or otherwise regulate gene expression. Such

regulatory elements include but are not limited to the cytomegalovirus hCMV immediate early gene, the early or late promoters of SV40 adenovirus, the lac system, the trp system, the TAC system, the TRC system, the major operator and promoter regions of phage A, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase, the promoters of acid phosphatase, and the promoters of the yeast α-mating factors.

Similarly, the nucleic acid can form part of a hybrid gene encoding additional polypeptide sequences, for example, a sequence that functions as a marker or reporter. Examples of marker and reporter genes include β-lactamase, chloramphenicol acetyltransferase (CAT), adenosine deaminase (ADA), aminoglycoside phosphotransferase (neo^r, $G418^r$), dihydrofolate reductase (DHFR), hygromycin-B-phosphotransferase (HPH), thymidine kinase (TK), lacZ (encoding β-galactosidase), and xanthine guanine phosphoribosyltransferase (XGPRT). As with many of the standard procedures associated with the practice of the invention, skilled artisans will be aware of additional useful reagents, for example, additional sequences that can serve the function of a marker or reporter. Generally, the hybrid polypeptide will include a first portion and a second portion; the first portion being one of the proteins encoded by genes corresponding to the SAGE tags listed in Fig. 7 (or a functional fragment of such a protein) and the second portion being, for example, one of the reporters described above or an Ig constant region or part of an Ig constant region, e.g., the CH2 and CH3 domains of IgG2a heavy chain. Other hybrids could include an antigenic tag or His tag to facilitate purification.

The expression systems that may be used for purposes of the invention include but are not limited to microorganisms such as bacteria (for example, *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA, or cosmid DNA expression vectors containing the nucleic acid molecules of the invention; yeast (for example, *Saccharomyces* and *Pichia*) transformed with recombinant yeast expression vectors containing the nucleic acid molecule of the invention; insect cell systems infected with recombinant virus expression vectors (for example, baculovirus) containing the nucleic acid molecule of the invention; plant cell systems infected with recombinant virus expression vectors (for example, cauliflower mosaic virus (CaMV) or tobacco mosaic virus (TMV)) or transformed with recombinant plasmid expression vectors (for example, Ti plasmid) containing any of the nucleotide sequences recited above; or mammalian cell systems (for example, COS, CHO, BHK, 293, VERO, HeLa, MDCK, WI38, and NIH 3T3 cells) harboring recombinant expression constructs containing promoters derived

from the genome of mammalian cells (for example, the metallothionein promoter) or from mammalian viruses (for example, the adenovirus late promoter and the vaccinia virus 7.5K promoter). Also useful as host cells are primary or secondary cells obtained directly from a mammal and transfected with a plasmid vector or infected with a viral vector.

Polypeptides and Polypeptide Fragments

The polypeptides of the invention include all those encoded by the nucleic acids described above and functional fragments of these polypeptides. The polypeptides embraced by the invention also include fusion proteins that contain either a full-length polypeptide, or a functional fragment thereof, fused to unrelated amino acid sequence. The unrelated sequences can be additional functional domains or signal peptides. The polypeptides can be any of those described above but with not more than 50 (e.g., not more than: 50; 40; 30; 25; 20;15; 12, 10; nine; eight; seven; six; five; four; three; two; or one) conservative substitution(s). Conservative substitutions typically include substitutions within the following groups: glycine and alanine; valine, isoleucine, and leucine; aspartic acid and glutamic acid; asparagine, glutamine, serine and threonine; lysine, histidine and arginine; and phenylalanine and tyrosine. All that is required of a polypeptide with one or more conservative substitutions is that it have at least 5% (e.g., at least: 5%; 10%; 20%; 30%; 40%; 50%; 60%; 70%; 80%; 90%; 95%; 98%; 99%; 100%; or more) of the activity (e.g., ability to inhibit proliferation of breast cancer cells) of the relevant wild-type, mature polypeptide.

Polypeptides of the invention and those useful for the invention can be purified from natural sources (e.g., blood, serum, plasma, tissues or cells such as normal breast or cancerous breast epithelial cells (of the luminal type), myoepithelial cells, leukocytes, or endothelial cells). Smaller peptides (less than 50 amino acids long) can also be conveniently synthesized by standard chemical means. In addition, both polypeptides and peptides can be produced by standard *in vitro* recombinant DNA techniques and *in vivo* transgenesis, using nucleotide sequences encoding the appropriate polypeptides or peptides. Methods well-known to those skilled in the art can be used to construct expression vectors containing relevant coding sequences and appropriate transcriptional/translational control signals. See, for example, the techniques described in Sambrook et al., *Molecular Cloning: A Laboratory Manual* (2nd Ed.)

[Cold Spring Harbor Laboratory, N.Y., 1989], and Ausubel et al., *Current Protocols in Molecular Biology* [Green Publishing Associates and Wiley Interscience, N.Y., 1989].

Polypeptides and fragments of the invention, and those useful for the invention, also include those described above, but modified for *in vivo* use by the addition, at the amino- and/or carboxyl-terminal ends, of a blocking agent to facilitate survival of the relevant polypeptide *in vivo*. This can be useful in those situations in which the peptide termini tend to be degraded by proteases prior to cellular uptake. Such blocking agents can include, without limitation, additional related or unrelated peptide sequences that can be attached to the amino and/or carboxyl terminal residues of the peptide to be administered. This can be done either chemically during the synthesis of the peptide or by recombinant DNA technology by methods familiar to artisans of average skill.

Alternatively, blocking agents such as pyroglutamic acid or other molecules known in the art can be attached to the amino and/or carboxyl terminal residues, or the amino group at the amino terminus or carboxyl group at the carboxyl terminus can be replaced with a different moiety. Likewise, the peptides can be covalently or noncovalently coupled to pharmaceutically acceptable "carrier" proteins prior to administration.

Also of interest are peptidomimetic compounds that are designed based upon the amino acid sequences of the functional peptide fragments. Peptidomimetic compounds are synthetic compounds having a three-dimensional conformation (i.e., a "peptide motif") that is substantially the same as the three-dimensional conformation of a selected peptide. The peptide motif provides the peptidomimetic compound with the ability to inhibit the pathogenesis of breast cancer cells in a manner qualitatively identical to that of the functional fragment from which the peptidomimetic was derived. Peptidomimetic compounds can have additional characteristics that enhance their therapeutic utility, such as increased cell permeability and prolonged biological half-life.

The peptidomimetics typically have a backbone that is partially or completely non-peptide, but with side groups that are identical to the side groups of the amino acid residues that occur in the peptide on which the peptidomimetic is based. Several types of chemical bonds, e.g., ester, thioester, thioamide, retroamide, reduced carbonyl, dimethylene and ketomethylene bonds, are known in the art to be generally useful substitutes for peptide bonds in the construction of protease-resistant peptidomimetics.

In the sections below, a "gene X" represents any of the genes listed in Tables 1-16; mRNA transcribed from gene X is referred to as "mRNA X"; protein encoded by gene X is referred to as "protein X"; and cDNA produced from mRNA X is referred to as "cDNA X". It is understood that, unless otherwise stated, descriptions containing these terms are applicable to any of the genes listed in Tables 1-16, mRNAs transcribed from such genes, proteins encoded by such genes, or cDNAs produced from the mRNAs.

Diagnostic assays

The invention features diagnostic assays. Such assays are based on the findings that: (a) certain genes are expressed at a higher level, or a lower level, in breast epithelial cancer cells (or non-epithelial cells within a relevant breast tumor) compared to normal cells of the same types; and (b) breast cancers of various grades and/or stages differ from each other in terms of the patterns of genes they express and in the levels at which they express them. These findings provide the bases for assays to diagnose breast cancer and to define the grade and/or stage of a breast cancer. Such assays can be used on their own or, preferably, in conjunction with other procedures to diagnose breast cancer and/or identify the grade and/or stage of progression of a breast cancer.

The diagnostic assays of the invention generally involve testing for levels of expression of one or a plurality of the genes listed in Tables 1-16. By testing for levels of expression in a cell of a plurality of genes, one obtains an "expression profile" of the cell.

In the assays of the invention either: (1) the presence of protein X or mRNA X in cells is tested for or their levels in cells are measured; or (2) the level of protein X is measured in a liquid sample such as a body fluid (e.g., urine, saliva, semen, blood, or serum or plasma derived from blood); a lavage such as a breast duct lavage, lung lavage, a gastric lavage, a rectal or colonic lavage, or a vaginal lavage; an aspirate such as a nipple aspirate; or a fluid such as a supernatant from a cell culture. In order to test for the presence, or measure the level, of mRNA X in cells, the cells can be lysed and total RNA can be purified or semi-purified from lysates by any of a variety of methods known in the art. Methods of detecting or measuring levels of particular mRNA transcripts are also familiar to those in the art. Such assays include, without limitation, hybridization assays using detectably labeled mRNA X-specific DNA or RNA probes

and quantitative or semi-quantitative RT-PCR methodologies employing appropriate mRNA X and cDNA X-specific oligonucleotide primers. Additional methods for quantitating mRNA in cell lysates include RNA protection assays and serial analysis of gene expression (SAGE). Alternatively, qualitative, quantitative, or semi-quantitative *in situ* hybridization assays can be carried out using, for example, tissue sections or unlysed cell suspensions, and detectably (e.g., fluorescently or enzyme) labeled DNA or RNA probes.

Methods of detecting or measuring the levels of a protein of interest in cells are known in the art. Many such methods employ antibodies (e.g., polyclonal antibodies or monoclonal antibodies (mAbs)) that bind specifically to the protein. In such assays, the antibody itself or a secondary antibody that binds to it can be detectably labeled. Alternatively, the antibody can be conjugated with biotin, and detectably labeled avidin (a protein that binds to biotin) can be used to detect the presence of the biotinylated antibody. Combinations of these approaches (including "multi-layer" assays) familiar to those in the art can be used to enhance the sensitivity of assays. Some of these assays (e.g., immunohistological methods or fluorescence flow cytometry) can be applied to histological sections or unlysed cell suspensions. The methods described below for detecting protein X in a liquid sample can also be used to detect protein X in cell lysates.

Methods of detecting protein X in a liquid sample (see above) basically involve contacting a sample of interest with an antibody that binds to protein X and testing for binding of the antibody to a component of the sample. In such assays the antibody need not be detectably labeled and can be used without a second antibody that binds to protein X. For example, by exploiting the phenomenon of surface plasmon resonance, an antibody specific for protein X bound to an appropriate solid substrate is exposed to the sample. Binding of protein X to the antibody on the solid substrate results in a change in the intensity of surface plasmon resonance that can be detected qualitatively or quantitatively by an appropriate instrument, e.g., a Biacore apparatus (Biacore International AB, Rapsgatan, Sweden).

Moreover, assays for detection of protein X in a liquid sample can involve the use, for example, of: (a) a single protein X-specific antibody that is detectably labeled; (b) an unlabeled protein X-specific antibody and a detectably labeled secondary antibody; or (c) a biotinylated protein X-specific antibody and detectably labeled avidin. In addition, as described above for detection of proteins in cells, combinations of these approaches (including "multi-layer" assays) familiar to those in the art can be used to enhance the sensitivity of assays. In these assays, the

sample or an (aliquot of the sample) suspected of containing protein X can be immobilized on a solid substrate such as a nylon or nitrocellulose membrane by, for example, "spotting" an aliquot of the liquid sample or by blotting of an electrophoretic gel on which the sample or an aliquot of the sample has been subjected to electrophoretic separation. The presence or amount of protein X on the solid substrate is then assayed using any of the above-described forms of the protein X-specific antibody and, where required, appropriate detectably labeled secondary antibodies or avidin.

The invention also features "sandwich" assays. In these sandwich assays, instead of immobilizing samples on solid substrates by the methods described above, any protein X that may be present in a sample can be immobilized on the solid substrate by, prior to exposing the solid substrate to the sample, conjugating a second ("capture") protein X-specific antibody (polyclonal or mAb) to the solid substrate by any of a variety of methods known in the art. In exposing the sample to the solid substrate with the second protein X-specific antibody bound to it, any protein X in the sample (or sample aliquot) will bind to the second protein X-specific antibody on the solid substrate. The presence or amount of protein X bound to the conjugated second protein X-specific antibody is then assayed using a "detection" protein X-specific antibody by methods essentially the same as those described above using a single protein X-specific antibody. It is understood that in these sandwich assays, the capture antibody should not bind to the same epitope (or range of epitopes in the case of a polyclonal antibody) as the detection antibody. Thus, if a mAb is used as a capture antibody, the detection antibody can be either: (a) another mAb that binds to an epitope that is either completely physically separated from or only partially overlaps with the epitope to which the capture mAb binds; or (b) a polyclonal antibody that binds to epitopes other than or in addition to that to which the capture mAb binds. On the other hand, if a polyclonal antibody is used as a capture antibody, the detection antibody can be either (a) a mAb that binds to an epitope to that is either completely physically separated from or partially overlaps with any of the epitopes to which the capture polyclonal antibody binds; or (b) a polyclonal antibody that binds to epitopes other than or in addition to that to which the capture polyclonal antibody binds. Assays which involve the used of a capture and detection antibody include sandwich ELISA assays, sandwich Western blotting assays, and sandwich immunomagnetic detection assays.

Suitable solid substrates to which the capture antibody can be bound include, without limitation, the plastic bottoms and sides of wells of microtiter plates, membranes such as nylon or nitrocellulose membranes, polymeric (e.g., without limitation, agarose, cellulose, or polyacrylamide) beads or particles. It is noted that protein X-specific antibodies bound to such beads or particles can also be used for immunoaffinity purification of protein X.

Methods of detecting or for quantifying a detectable label depend on the nature of the label and are known in the art. Appropriate labels include, without limitation, radionuclides (e.g., ^{125}I, ^{131}I, ^{35}S, ^{3}H, ^{32}P, ^{33}P, or ^{14}C), fluorescent moieties (e.g., fluorescein, rhodamine, or phycoerythrin), luminescent moieties (e.g., Qdot™ nanoparticles supplied by the Quantum Dot Corporation, Palo Alto, CA), compounds that absorb light of a defined wavelength, or enzymes (e.g., alkaline phosphatase or horseradish peroxidase). The products of reactions catalyzed by appropriate enzymes can be, without limitation, fluorescent, luminescent, or radioactive or they may absorb visible or ultraviolet light. Examples of detectors include, without limitation, x-ray film, radioactivity counters, scintillation counters, spectrophotometers, colorimeters, fluorometers, luminometers, and densitometers.

In assays, for example, to diagnose breast cancer, the level of protein X in, for example, serum (or a breast cell) from a patient suspected of having, or at risk of having, breast cancer is compared to the level of protein X in sera (or breast cells) from a control subject (e.g., a subject not having breast cancer) or the mean level of protein X in sera (or breast cells) from a control group of subjects (e.g., subjects not having breast cancer). A significantly higher level, or lower level (depending on whether the gene of interest is expressed at higher or lower level in breast cancer or associated stromal cells), of protein X in the serum (or breast cells) of the patient relative to the mean level in sera (or breast cells) of the control group would indicate that the patient has breast cancer. Alternatively, if a sample of the subject's serum (or breast cells) that was obtained at a prior date at which the patient clearly did not have breast cancer is available, the level of protein in the test serum (or breast cell) sample can be compared to the level in the prior obtained sample. A higher level, or lower level (depending on whether the gene of interest is expressed at higher or lower level in breast cancer or associated stromal cells) in the test serum (or breast cell) sample would be an indication that the patient has breast cancer.

Moreover, a test expression profile of a gene in a test cell (or tissue) can be compared to control expression profiles of control cells (or tissues) previously established to be of defined

category (e.g., DCIS grade, breast cancer stage, or state of differentiation). The category of the the test cell (or tissue) will be that of the control cell (or tissue) whose expression profile the test cell's (or tissue's) expression profile most closely resembles. These expression profile comparison assays can be used to compare any of the normal breast tissue with any stage and/or grade of breast cancer recited herein and/or to compare between breast cancer grades and stages. The genes analyzed can be any of those listed in Tables 1-16 and the number of genes analyzed can be any number, i.e. one or more. Generally, at least two (e.g., at least: two; three; four; five; six; seven; eight; nine; ten; 11; 12; 13; 14; 15; 17; 18; 20; 23; 25; 30; 35; 40; 45; 50; 60; 70; 80; 90; 100; 120; 150; 200; 250; 300; 350; 400; 450; 500; or more) genes will be analyzed. It is understood that the genes analyzed will include at least one of those listed herein but can also include others not listed herein.

One of skill in the art will appreciate from this description how similar "test level" versus "control level" comparisons can be made between other test and control samples described herein.

It is noted that the patients and control subjects referred to above need not be human patients. They can be for example, non-human primates (e.g., monkeys), horses, sheep, cattle, goats, pigs, dogs, guinea pigs, hamsters, rats, rabbits or mice.

Methods of Inhibiting Expression of Genes

Also included in the invention are methods of inhibiting expression of the genes listed in Tables 2-10, 15, and 16 in cells, e.g., breast epithelial cancer cells and/or stromal cells (e.g., leukocytes, myoepithelial cells, myofibroblasts, endothelial cells, or fibroblasts) in a tumor containing the cancer cells; such methods are applicable where the expression of protein X in breast cancer cells, or stromal cells in a breast tumor, is higher than in corresponding normal cells. These methods can also be adapted to inhibit expression of a receptor for a ligand protein X. One such method involves introducing into a cell (a) an antisense oligonucleotide or (b) a nucleic acid comprising a transcriptional regulatory element (TRE) operably linked to a nucleic sequence that is transcribed in the cell into an antisense RNA. The antisense oligonucleotide and the antisense RNA hybridize to a mRNA X molecule (or mRNA molecule encoding a receptor for a ligand protein X) and have the effect in the cell of inhibiting expression of protein X (or receptor for protein X) in the cell. Inhibiting protein X/protein X receptor expression in the

breast cancer cells or stromal cells can inhibit pathogenesis of breast cancer cells. The method can thus be useful in inhibiting pathogenesis of a breast cancer cell and can be applied to the therapy of breast cancer, e.g., DCIS, invasive breast cancer, or metastatic breast cancer.

Antisense compounds are generally used to interfere with protein expression either by, for example, interfering directly with translation of a target mRNA molecule, by RNAse-H-mediated degradation of the target mRNA, by interference with 5' capping of mRNA, by prevention of translation factor binding to the target mRNA by masking of the 5' cap, or by inhibiting of mRNA polyadenylation. The interference with protein expression arises from the hybridization of the antisense compound with its target mRNA. A specific targeting site or a target mRNA of interest for interaction with an antisense compound is chosen. Thus, for example, for modulation of polyadenylation a preferred target site on an mRNA target is a polyadenylation signal or a polyadenylation site. For diminishing mRNA stability or degradation, destabilizing sequence are preferred target sites. Once one or more target sites have been identified, oligonucleotides are chosen which are sufficiently complementary to the target site (i.e., hybridize sufficiently well under physiological conditions and with sufficient specificity) to give the desired effect.

With respect to this invention, the term "oligonucleotide" refers to an oligomer or polymer of RNA, DNA, or a mimetic of either. The term includes oligonucleotides composed of naturally-occurring nucleobases, sugars, and covalent internucleoside (backbone) linkages. The normal linkage or backbone of RNA and DNA is a 3' to 5' phosphodiester bond. The term also refers however to oligonucleotides composed entirely of, or having portions containing, non-naturally occurring components which function in a similar manner to the oligonucleotides containing only naturally-occurring components. Such modified substituted oligonucleotides are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for target sequence, and increased stability in the presence of nucleases. In the mimetics, the core base (pyrimidine or purine) structure is generally preserved but (1) the sugars are either modified or replaced with other components and/or (2) the inter-nucleobase linkages are modified. One class of nucleic acid mimetic that has proven to be very useful is referred to as protein nucleic acid (PNA). In PNA molecules the sugar backbone is replaced with an amide-containing backbone, in particular an aminoethylglycine backbone. The bases are retained and are bound directly to the aza nitrogen atoms of the amide portion of the

backbone. PNA and other mimetics useful in the instant invention are described in detail in U.S. Patent No. 6,210,289, which is incorporated herein by reference in its entirety.

The antisense oligomers to be used in the methods of the invention generally comprise about 8 to about 100 (e.g., about 14 to about 80 or about 14 to about 35) nucleobases (or nucleosides where the nucleobases are naturally occurring) .

The antisense oligonucleotides can themselves be introduced into a cell or an expression vector containing a nucleic sequence (operably linked to a TRE) encoding the antisense oligonucleotide can be introduced into the cell. In the latter case, the oligonucleotide produced by the expression vector is an RNA oligonucleotide and the RNA oligonucleotide will be composed entirely of naturally occurring components.

The methods of the invention can be *in vitro* or *in vivo*. *In vitro* applications of the methods can be useful, for example, in basic scientific studies on cancer cell pathogenesis, e.g., cancer cell proliferation and/or cell survival. In such *in vitro* methods, appropriate cells (see above), can be incubated for various lengths of time with (a) the antisense oligonucleotides or (b) expression vectors containing nucleic acid sequences encoding the antisense oligonucleotides at a variety of concentrations. Other incubation conditions known to those in art (e.g., temperature or cell concentration) can also be varied. Inhibition of protein X expression can be tested by methods known to those in the art. However, the methods of the invention will preferably be *in vivo*.

As used herein, "prophylaxis" can mean complete prevention of the symptoms of a disease (e.g., breast cancer such as DCIS), a delay in onset of the symptoms of a disease, or a lessening in the severity of subsequently developed disease symptoms. "Prevention" should mean that symptoms of the disease (e.g., breast cancer) are essentially absent. As used herein, "therapy" can mean a complete abolishment of the symptoms of a disease or a decrease in the severity of the symptoms of the disease. As used herein, a "protective" regimen is a regimen that is prophylactic and/or therapeutic.

The antisense methods are generally useful for cancer cells (e.g., a breast cancer cell) cancer cell pathogenesis-inhibiting therapy or prophylaxis. They can be administered to mammalian subjects (e.g., human breast cancer patients) alone or in conjunction with other drugs and/or radiotherapy.

Where antisense oligonucleotides *per se* are administered, they can be suspended in a pharmaceutically-acceptable carrier (e.g., physiological saline) and administered orally, intrarectally, intravaginally, intranasally, intragastrically, intratracheally, or intrapulmonarily, or injected subcutaneously, intramuscularly, intrathecally, intraperitoneally, intravenously. They can also be delivered directly to tumor cells, e.g., to a tumor or a tumor bed following surgical excision of the tumor, in order to kill any remaining tumor cells. The dosage required depends on the choice of the route of administration; the nature of the formulation; the nature of the patient's illness; the subject's size, weight, surface area, age, and sex; other drugs being administered; and the judgment of the attending physician. Suitable dosages are generally in the range of 0.01 mg/kg – 100 mg/kg. Wide variations in the needed dosage are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization as is well understood in the art. Administrations can be single or multiple (e.g., 2-, 3-, 4-, 6-, 8-, 10-, 20-, 50-,100-, 150-, or more fold). Encapsulation of the polypeptide in a suitable delivery vehicle (e.g., polymeric microparticles or implantable devices) may increase the efficiency of delivery, particularly for oral delivery.

Where an expression vector containing a nucleic sequence (operably linked to a TRE) encoding the antisense oligonucleotide is administered to a subject, expression of the coding sequence can be directed to any cell in the body of the subject. However, expression will preferably be directed to cells in a tumor containing the cancer cells or cells in the immediate vicinity of the cancer cells whose pathogenesis it is desired to inhibit. Expression of the coding sequence can be directed to the tumor cells themselves. This can be achieved by, for example, the use of polymeric, biodegradable microparticle or microcapsule delivery devices known in the art.

Another way to achieve uptake of the nucleic acid is using liposomes, prepared by standard methods. The vectors can be incorporated alone into these delivery vehicles or co-incorporated with tissue-specific or tumor-specific antibodies. Alternatively, one can prepare a molecular conjugate composed of a plasmid or other vector attached to poly-L-lysine by electrostatic or covalent forces. Poly-L-lysine binds to a ligand that can bind to a receptor on

target cells [Cristiano et al. (1995), J. Mol. Med. 73:479]. Alternatively, tissue-specific targeting can be achieved by the use of tissue-specific transcriptional/translational regulatory elements (TRE), e.g., promoters and enhancers, which are known in the art. Delivery of "naked DNA" (i.e., without a delivery vehicle) to an intramuscular, intradermal, or subcutaneous site is another means to achieve *in vivo* expression.

Enhancers provide expression specificity in terms of time, location, and level. Unlike a promoter, an enhancer can function when located at variable distances from the transcription initiation site, provided a promoter is present. An enhancer can also be located downstream of the transcription initiation site. To bring a coding sequence under the control of a promoter, it is necessary to position the translation initiation site of the translational reading frame of the peptide or polypeptide between one and about fifty nucleotides downstream (3') of the promoter. The coding sequence of the expression vector is operatively linked to a transcription terminating region.

The transcriptional/translational regulatory elements referred to above include, but are not limited to, inducible and non-inducible promoters, enhancers, operators and other elements that are known to those skilled in the art and that drive or otherwise regulate gene expression. Examples of such regulatory elements are provided above in the section on Nucleic Acids.

Suitable expression vectors include plasmids and viral vectors such as herpes viruses, retroviruses, vaccinia viruses, attenuated vaccinia viruses, canary pox viruses, adenoviruses and adeno-associated viruses, among others.

Polynucleotides can be administered in a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are biologically compatible vehicles that are suitable for administration to a human, e.g., physiological saline or liposomes. A therapeutically effective amount is an amount of the polynucleotide that is capable of producing a medically desirable result (e.g., decreased proliferation and or survival of breast cancer cells) in a treated animal. As is well known in the medical arts, the dosage for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Dosages will vary, but a preferred dosage for administration of polynucleotide is from approximately 10^6 to approximately 10^{12} copies of the polynucleotide molecule. This dose

can be repeatedly administered, as needed. Routes of administration can be any of those listed above.

Double-stranded interfering RNA (RNAi) homologous to mRNA X can also be used to reduce expression of protein X in a cell. See, e.g., Fire et al. (1998) Nature 391:806-811; Romano and Masino (1992) Mol. Microbiol. 6:3343-3353; Cogoni et al. (1996) EMBO J. 15:3153-3163; Cogoni and Masino (1999) Nature 399:166-169; Misquitta and Paterson (1999) Proc. Natl. Acad. Sci. USA 96:1451-1456; and Kennerdell and Carthew (1998) Cell 95:1017-1026.

The sense and anti-sense RNA strands of RNAi can be individually constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, each strand can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecule or to increase the physical stability of the duplex formed between the sense and anti-sense strands, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides. The sense or anti-sense strand can also be produced biologically using an expression vector into which a target protein X sequence (full-length or a fragment) has been subcloned in a sense or anti-sense orientation. The sense and anti-sense RNA strands can be annealed *in vitro* before delivery of the dsRNA to any of cancer cells disclosed herein. Alternatively, annealing can occur *in vivo* after the sense and anti-sense strands are sequentially delivered to the cancer cells.

Double-stranded RNA interference can also be achieved by introducing into cancer cells a polynucleotide from which sense and anti-sense RNAs can be transcribed under the direction of separate promoters, or a single RNA molecule containing both sense and anti-sense sequences can be transcribed under the direction of a single promoter.

Also useful for inhibiting expression of gene X are "small molecule" inhibitors of gene expression. Such small molecules are useful for inhibiting a function of protein X or a downstream activity initiated by or via protein X. For example, quinazoline compounds are useful in inhibiting tyrosine kinase activity that, for example, is stimulated by binding of a ligand to one of epidermal growth factor receptors (EGFR), e.g., erbB1 or erbB2. Small molecules of interest include, without limitation, small non-nucleic acid organic molecules, small inorganic molecules, peptides, peptoids, peptidomimetics, non-naturally occurring nucleotides, and small nucleic acids (e.g., RNAi or antisense oligonucleotides). Generally, small molecules have

molecular weights of less than 10 kDa (e.g., less than: 10 kDa; 9 kDa; 8 kDa; 7 kDa; 6 kDa; 5 kDa; 4 kDa; 3 kDa; 2 kDa; or 1 kDa).

Other methods of interest include the recently described degrakine and intrakine techniques [Coffield et al. (2003) Nat. Biotech. 21:1321-1327; Chen et al. (1997) Nat. Med. 3:1110-1116], which result in inhibition of expression, on the surface of a target cell (e.g., a breast cancer cell), of a receptor for a ligand protein (e.g., a soluble ligand such as a cytokine, chemokine, or growth factor or a ligand on the surface of another cell). By inhibiting expression of the receptor on the target cell, responsiveness of the target cell to the ligand protein is inhibited or, optimally, prevented.

In the degrakine methodology, a fusion protein is used to inhibit cell surface expression of a receptor for a ligand protein X of interest (e.g., a receptor for CXCL14), the receptor being on the surface of a target cell of interest (e.g., a breast cancer cell). The fusion protein is a fusion between (a) a ligand protein X (or a fragment of the protein X ligand that retains the ability to bind to the receptor for the protein X ligand) and (b) the HIV-1 Vpu protein. The target cell of interest is contacted *in vivo* or *in vitro* with an expression vector (e.g., a viral vector such as any of those disclosed herein) expressing the fusion protein. After entry of the expression vector into the cell, the fusion protein is produced in the cytoplasm of the target cell. The fusion protein, due to the activity of the Vpu protein, then migrates to the endoplasmic reticulum (ER) of the target cell where it can bind to recently translated ligand protein X receptor molecules and inhibit or, optimally, prevent translocation of the receptor molecules to the surface of the target cell. Moreover, it is believed that the Vpu component of the fusion protein bound to newly made receptor molecules targets the receptor molecules for degradation by proteasomes within the target cell [Coffield et al. (2003)].

Intrakine methodologies are conceptually similar to the degrakine methodology. Instead of the Vpu protein, a signal sequence that serves to direct proteins containing it to the ER (e.g., the four amino acid KDEL (SEQ ID NO:1956) sequence) is fused to the ligand protein X (or a fragment of the protein X ligand that retains the ability to bind to the receptor for the ligand protein X) [Coffield et al. (2003); Chen et al. (1997)].

The degrakine and intrakine methodologies can be modified as follows. The fusion protein itself can be contacted (*in vivo* or *in vitro*) with a target cell expressing a surface receptor for the ligand protein X. The fusion protein can then, e.g., by binding to such a receptor, enter

the cytoplasm of the target cell. The fusion protein then, as in the vector-mediated method described above, migrates to the ER of the target cell and inhibits translocation of the receptor to the target cell surface.

One of skill in the art will appreciate that RNAi, small molecule, and degrakine/intrakine methods can be, as for the antisense methods described above, *in vitro* and *in vivo*. Moreover, methods and conditions of delivery for RNAi, small molecule, and degrakine/intrakine methods can be applied are the same as those for antisense oligonucleotides.

The antisense, RNAi, small molecule, and degrakine/intrakine methods of the invention can be applied to a wide range of species, e.g., humans, non-human primates, horses, cattle, pigs, sheep, goats, dogs, cats, rabbits, guinea pigs, hamsters, rats, and mice.

Passive Immunoprotection

The methods described in this section are applicable where the expression of protein X in breast cancer cells, or stromal cells in a breast tumor, is higher than in corresponding normal cells.

As used herein, "passive immunoprotection" means administration of one or more protein X-binding agents to a subject that has, is suspected of having, or is at risk of having a breast cancer, e.g., a DCIS, an invasive breast cancer, or a metastatic breast cancer. Thus, passive immunoprotection can be prophylactic and/or therapeutic. As used herein, "protein X-binding agents" are agents that bind to protein X and thereby inhibit the ability of protein X to enhance pathogenesis of breast cancer cells. It is understood that the term "inhibit" includes "completely inhibit" and "partially inhibit." Protein X-binding agents can be, for example, a soluble (i.e., not cell-bound) full length form (or fragment such as a fragment lacking a transmembrane domain) of a receptor for protein X (where protein X is a ligand), a soluble, non-agonist form (or fragment of a ligand for protein X (where protein X is a receptor), or a non-agonist, antibody specific for protein X. Other useful agents include non-agonist molecules that bind to a receptor for a protein X (i.e., protein X receptor-binding agents). Such protein X receptor-binding agents include non-agonist antibodies specific for a protein X receptor and non-agonist fragments of a protein X that retain the ability to bind to the receptor for protein X. A protein X-binding agent (or a protein X receptor-binding agent) useful for the invention has the capacity to inhibit the ability of protein X to enhance the pathogenesis (e.g., proliferation and/or survival) of the breast

cancer cells by at least 20% (e.g., at least: 20%; 30%; 40%; 50%; 60%; 70%; 80%; 90%; 95%; 98%; 99%; 99.5%, or even 100%).

Antibodies can be polyclonal or monoclonal antibodies; methods for producing both types of antibody are known in the art. The antibodies can be of any class (e.g., IgM, IgG, IgA, IgD, or IgE) and be generated in any of the species recited herein. They are preferably IgG antibodies. Recombinant antibodies, such as chimeric and humanized monoclonal antibodies comprising both human and non-human portions, can also be used in the methods of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example, using methods described in Robinson et al., International Patent Publication PCT/US86/02269; Akira et al., European Patent Application 184,187; Taniguchi, European Patent Application 171,496; Morrison et al., European Patent Application 173,494; Neuberger et al., PCT Application WO 86/01533; Cabilly et al., U.S. Patent No. 4,816,567; Cabilly et al., European Patent Application 125,023; Better et al. (1988) Science 240, 1041-43; Liu et al. (1987) J. Immunol. 139, 3521-26; Sun et al. (1987) PNAS 84, 214-18; Nishimura et al. (1987) Canc. Res. 47, 999-1005; Wood et al. (1985) Nature 314, 446-49; Shaw et al. (1988) J. Natl. Cancer Inst. 80, 1553-59; Morrison, (1985) Science 229, 1202-07; Oi et al. (1986) BioTechniques 4, 214; Winter, U.S. Patent No. 5,225,539; Jones et al. (1986) Nature 321, 552-25; Veroeyan et al. (1988) Science 239, 1534; and Beidler et al. (1988) J. Immunol. 141, 4053-60.

Also useful for the invention are antibody fragments and derivatives that contain at least the functional portion of the antigen-binding domain of an antibody. Antibody fragments that contain the binding domain of the molecule can be generated by known techniques. Such fragments include, but are not limited to: $F(ab')_2$ fragments that can be produced by pepsin digestion of antibody molecules; Fab fragments that can be generated by reducing the disulfide bridges of $F(ab')_2$ fragments; and Fab fragments that can be generated by treating antibody molecules with papain and a reducing agent. See, e.g., National Institutes of Health, 1 Current Protocols In Immunology, Coligan et al., ed. 2.8, 2.10 (Wiley Interscience, 1991). Antibody fragments also include Fv fragments, i.e., antibody products in which there are few or no constant region amino acid residues. A single chain Fv fragment (scFv) is a single polypeptide chain that includes both the heavy and light chain variable regions of the antibody from which the scFv is derived. Such fragments can be produced, for example, as described in U.S. Patent

No. 4,642,334, which is incorporated herein by reference in its entirety. For a human subject, the antibody can be a "humanized" version of a monoclonal antibody originally generated in a different species.

The invention includes antibodies specific for the proteins encoded by genes corresponding to the SAGE tags listed in Fig. 7. The antibodies can be of any of the types and classed referred to herein.

Protein X-binding (or protein X receptor-binding) agents can be administered to any of the species listed herein. The binding agents will preferably, but not necessarily, be of the same species as the subject to which they are administered. A single polyclonal or monoclonal antibody can be administered, or two or more (e.g., two, three, four, five, six, seven, eight, nine, ten, 12, 14, 16, 18, or 20) polyclonal antibodies or monoclonal antibodies can be given. The binding agents can be administered to subjects prior to, subsequently to, or at the same time as the protein X-expression inhibitors (see above).

The dosage of protein X/protein X receptor-binding agents required depends on the route of administration, the nature of the formulation, the nature of the patient's illness, the subject's size, weight, surface area, age, and sex, other drugs being administered, and the judgment of the attending physician. Suitable dosages are in the range of 0.01-100.0 mg/kg. The protein X/protein X receptor-binding agents can be administered by any of the routes disclosed herein, but will generally be administered intravenously, intramuscularly, or subcutaneously. Wide variations in the needed dosage are to be expected in view of the variety of protein X/protein X receptor-binding agents (e.g., protein X-specific antibodies) available and the differing efficiencies of various routes of administration. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art. Administrations can be single or multiple (e.g., 2- or 3-, 4-, 6-, 8-, 10-, 20-, 50-, 100-, 150-, or more fold).

Methods to test whether a compound or antibody is therapeutic for, or prophylactic against, a particular disease are known in the art. Where a therapeutic effect is being tested, a test population displaying symptoms of the disease (e.g., breast cancer such as DCIS) is treated with a protein X/protein X receptor expression inhibitor or protein X/protein X receptor-binding agent using any of the above-described strategies. A control population, also displaying symptoms of the disease, is treated, using the same methodology, with a placebo. Disappearance

or a decrease of the disease symptoms in the test subjects would indicate that the compound or antibody was an effective therapeutic agent. By applying the same strategies to subjects at risk of having the disease, the compounds and antibodies can be tested for efficacy as prophylactic agents. In this situation, prevention of or delay in onset of disease symptoms is tested.

Methods of Inhibiting Pathogenesis of a Cancer Cell

Such methods are applicable where the expression of protein X in breast cancer cells, or stromal cells in a breast tumor, is lower than in corresponding normal cells (see Tables 1, 3-10, and 15). These methods involve contacting a breast cancer cell with a protein X, or a functional fragment thereof, in order to inhibit pathogenesis (e.g., proliferation or survival) of the cancer cell. Such polypeptides or functional fragments can have amino acid sequences identical to wild-type sequences or they can contain not more than 50 (e.g., not more than: 50; 40; 30; 25; 20; 15; 12; 10; nine; eight; seven; six; five; four; three; two; or one) conservative amino acid substitution(s). Alleles of the polypeptides encoded by listed in Tables 1, 3-10, and 15 are also useful for the invention.

The methods can be performed *in vitro*, *in vivo*, or *ex vivo*. *In vitro* application of protein X can be useful, for example, in basic scientific studies of tumor cell biology, e.g., studies on cancer cell proliferation, survival, invasion, metastasis, or escape from immunological effector mechanisms or studies on angiogenesis. In addition, protein X and the polynucleotides encoding protein X (DNA and/or RNA) can be used as "positive controls" in diagnostic assays (see below). However, the methods of the invention will preferably be *in vivo* or *ex vivo* (see below).

Protein X and variants thereof are generally useful as cancer cell (e.g., breast cancer cell) pathogenesis-inhibiting therapeutics. They can be administered to mammalian subjects (e.g., human breast cancer patients) alone or in conjunction with such drugs and/or radiotherapy.

These methods of the invention can be applied to a wide range of species, e.g., humans, non-human primates, horses, cattle, pigs, sheep, goats, dogs, cats, rabbits, guinea pigs, hamsters, rats, and mice.

In Vivo Approaches

In one *in vivo* approach, protein X (or a functional fragment thereof) itself is administered to the subject. Generally, the compounds of the invention will be suspended in a pharmaceutically-acceptable carrier (e.g., physiological saline) and administered orally or by

intravenous infusion, or injected subcutaneously, intramuscularly, intrathecally, intraperitoneally, intrarectally, intravaginally, intranasally, intragastrically, intratracheally, or intrapulmonarily. They are preferably delivered directly to tumor cells, e.g., to a tumor or a tumor bed following surgical excision of the tumor, in order to kill any remaining tumor cells. The dosage required depends on the choice of the route of administration; the nature of the formulation; the nature of the patient's illness; the subject's size, weight, surface area, age, and sex; other drugs being administered; and the judgment of the attending physician. Suitable dosages are in the range of 0.01-100.0 μg/kg. Wide variations in the needed dosage are to be expected in view of the variety of polypeptides and fragments available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by i.v. injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization as is well understood in the art. Administrations can be single or multiple (e.g., 2-, 3-, 4-, 6-, 8-, 10-, 20-, 50-,100-, 150-, or more fold). Encapsulation of the polypeptide in a suitable delivery vehicle (e.g., polymeric microparticles or implantable devices) may increase the efficiency of delivery, particularly for oral delivery.

Alternatively, a polynucleotide containing a nucleic acid sequence encoding protein X or functional fragment thereof can be delivered to breast cancer cells in a mammal. Expression of the coding sequence will preferably be directed to lymphoid tissue of the subject by, for example, delivery of the polynucleotide to the lymphoid tissue. Expression of the coding sequence can be directed to any cell in the body of the subject. However, expression will preferably be directed to cells (e.g., stromal cells) in a tumor containing, or in the vicinity of, the cancer cells whose proliferation it is desired to inhibit. In certain embodiments, expression of the coding sequence can be directed to the tumor cells themselves. This can be achieved by, for example, the use of polymeric, biodegradable microparticle or microcapsule delivery devices known in the art.

Another way to achieve uptake of the nucleic acid is using liposomes (see section above on Methods of Inhibiting Expression of Genes).

In the relevant polynucleotides (e.g., expression vectors), the nucleic acid sequence encoding protein X or functional fragment of interest with an initiator methionine and optionally a targeting sequence is operatively linked to a promoter or enhancer-promoter combination.

Short amino acid sequences can act as signals to direct proteins to specific intracellular compartments. Such signal sequences are described in detail in U.S. Patent No. 5,827,516, which is incorporated herein by reference in its entirety.

Appropriate enhancers, vectors, and methods of administration of polynucleotides are described above in the section on Methods of Inhibiting Gene Expression.

Ex Vivo Approaches

An *ex vivo* strategy can involve transfecting or transducing cells obtained from the subject with a polynucleotide encoding protein X or functional fragment-encoding nucleic acid sequences described above. The transfected or transduced cells are then returned to the subject. The cells can be any of a wide range of types including, without limitation, hemopoietic cells (including leukocytes) (e.g., bone marrow cells, macrophages, monocytes, dendritic cells, T cells, or B cells), fibroblasts, epithelial cells, endothelial cells, keratinocytes, or muscle cells. Such cells act as a source of the protein X or functional fragment for as long as they survive in the subject. Alternatively, tumor cells, preferably obtained from the subject but potentially from an individual other than the subject, can be transfected or transformed by a vector encoding a protein X or functional fragment thereof. The tumor cells, preferably treated with an agent (e.g., ionizing irradiation) that ablates their proliferative capacity, are then introduced into the patient, where they secrete exogenous protein X.

The *ex vivo* methods include the steps of harvesting cells from a subject, culturing the cells, transducing them with an expression vector, and maintaining the cells under conditions suitable for expression of the protein polypeptide or functional fragment. These methods are known in the art of molecular biology. The transduction step is accomplished by any standard means used for *ex vivo* gene therapy, including calcium phosphate, lipofection, electroporation, viral infection, and biolistic gene transfer. Alternatively, liposomes or polymeric microparticles can be used. Cells that have been successfully transduced can then be selected, for example, for expression of the coding sequence or of a drug resistance gene. The cells may then be lethally irradiated (if desired) and injected or implanted into the patient.

Arrays and Uses Thereof

The invention features an array that includes a substrate having a plurality of addresses. At least one address of the plurality includes a capture probe that binds specifically to a nucleic

acid X or a protein X. The array can have a density of at least, or less than, 10, 20 50, 100, 200, 500, 700, 1,000, 2,000, 5,000 or 10,000 or more addresses/cm^2, and ranges between. In a preferred embodiment, the plurality of addresses includes at least 10, 100, 500, 1,000, 5,000, 10,000, 50,000 addresses. In a preferred embodiment, the plurality of addresses includes equal to or less than 10, 100, 500, 1,000, 5,000, 10,000, or 50,000 addresses. The substrate can be a two-dimensional substrate such as a glass slide, a wafer (e.g., silica or plastic), a mass spectroscopy plate, or a three-dimensional substrate such as a gel pad. Addresses in addition to address of the plurality can be disposed on the array.

In one embodiment, at least one address of the plurality includes a nucleic acid capture probe that hybridizes specifically to a nucleic acid X, e.g., the sense or anti-sense strand. Nucleic acids of interest include, without limitation, all or part of any of the genes identified by the tags listed in Tables 1-16, all or part of mRNAs transcribed from such genes, or all or part of cDNA produced from such mRNA. Useful probes can, for example, be or contain the nucleotide sequences of the tags listed in Tables 1-5, 7-10, 15 and 16. Each address of the subset can include a capture probe that hybridizes to a different region of a nucleic acid. Each address of the subset is unique, overlapping, and complementary to a different variant of gene X (e.g., an allelic variant, or all possible hypothetical variants). The array can be used to sequence gene X, mRNA X, or cDNA X by hybridization (see, e.g., U.S. Patent No. 5,695,940).

An array can be generated by any of a variety of methods. Appropriate methods include, e.g., photolithographic methods (see, e.g., U.S. Patent Nos. 5,143,854; 5,510,270; and 5,527,681), mechanical methods (e.g., directed-flow methods as described in U.S. Patent No. 5,384,261), pin-based methods (e.g., as described in U.S. Pat. No. 5,288,514), and bead-based techniques (e.g., as described in PCT US/93/04145).

In another embodiment, at least one address of the plurality includes a polypeptide capture probe that binds specifically to protein X or fragment thereof. The polypeptide can be a naturally-occurring interaction partner of protein X, e.g., a ligand for protein X where protein X if a receptor or a receptor for protein X where protein X is ligand. Preferably, the polypeptide is an antibody, e.g., an antibody specific for protein X, such as a polyclonal antibody, a monoclonal antibody, or a single-chain antibody.

In another aspect, the invention features a method of analyzing the expression of gene X. The method includes providing an array as described above; contacting the array with a sample

and detecting binding of a nucleic acid X or protein X to the array. In one embodiment, the array is a nucleic acid array. Optionally the method further includes amplifying nucleic acid from the sample prior or during contact with the array.

In another embodiment, the array can be used to assay gene expression in a tissue to ascertain tissue specificity of genes in the array, particularly the expression of gene X. If a sufficient number of diverse samples is analyzed, clustering (e.g., hierarchical clustering, k-means clustering, Bayesian clustering and the like) can be used to identify other genes which are co-regulated with gene X. For example, the array can be used for the quantitation of the expression of multiple genes. Thus, not only tissue specificity, but also the level of expression of a battery of genes in the tissue is ascertained. Quantitative data can be used to group (e.g., cluster) genes on the basis of their tissue expression *per se* and level of expression in that tissue.

For example, array analysis of gene expression can be used to assess the effect of cell-cell interactions on gene X expression. A first tissue can be perturbed and nucleic acid from a second tissue that interacts with the first tissue can be analyzed. In this context, the effect of one cell type on another cell type in response to a biological stimulus can be determined, e.g., to monitor the effect of cell-cell interaction at the level of gene expression.

Moreover, cells can be contacted with a therapeutic agent. The expression profile of the cells is determined using the array, and the expression profile is compared to the profile of like cells not contacted with the agent. For example, the assay can be used to determine or analyze the molecular basis of an undesirable effect of the therapeutic agent. If an agent is administered therapeutically to treat one cell type but has an undesirable effect on another cell type, the invention provides an assay to determine the molecular basis of the undesirable effect and thus provides the opportunity to co-administer a counteracting agent or otherwise treat the undesired effect. Similarly, even within a single cell type, undesirable biological effects can be determined at the molecular level. Thus, the effects of an agent on expression of other than the target gene can be ascertained and counteracted.

In another embodiment, the array can be used to monitor expression of one or more genes in the array with respect to time. For example, samples obtained from different time points can be probed with the array. Such analysis can identify and/or characterize the development of a gene X-associated disease or disorder (e.g., breast cancer such as invasive breast cancer); and processes, such as a cellular transformation associated with a gene X-associated disease or

disorder. The method can also evaluate the treatment and/or progression of a gene X-associated disease or disorder

The array is also useful for ascertaining differential expression patterns of one or more genes in normal and abnormal (e.g., malignant) cells. This provides a battery of genes (e.g., including gene X) that could serve as a molecular target for diagnosis or therapeutic intervention.

In another aspect, the invention features an array having a plurality of addresses. Each address of the plurality includes a unique polypeptide. At least one address of the plurality has disposed thereon a protein or fragment thereof. Methods of producing polypeptide arrays are described in the art [e.g., in De Wildt et al. (2000) Nature Biotech. 18:989-994; Lueking et al. (1999) Anal. Biochem. 270:103-111; Ge, H. (2000) Nucleic Acids Res. 28 e3:I-VII; MacBeath, G., and Schreiber, S.L. (2000) Science 289:1760-1763; and WO 99/51773A1]. In a preferred embodiment, each addresses of the plurality has disposed thereon a polypeptide at least 60, 70, 80, 85, 90, 95, or 99 % identical to protein X or fragment thereof. For example, multiple variants of protein X (e.g., encoded by allelic variants, site-directed mutants, random mutants, or combinatorial mutants) can be disposed at individual addresses of the plurality. Addresses in addition to the address of the plurality can be disposed on the array.

The polypeptide array can be used to detect a protein X-binding compound, e.g., an antibody in a sample from a subject with specificity for protein X or the presence of a protein X-binding protein or ligand.

The array is also useful for ascertaining the effect of the expression of a gene on the expression of other genes in the same cell or in different cells (e.g., ascertaining the effect of gene X expression on the expression of other genes). This provides, for example, for a selection of alternate molecular targets for therapeutic intervention if the ultimate or downstream target cannot be regulated.

In another aspect, the invention features a method of analyzing a plurality of probes. The method is useful, e.g., for analyzing gene expression. The method includes: providing a first two dimensional array having a plurality of addresses, each address (of the plurality) being positionally distinguishable from each other address (of the plurality) having a unique capture probe, e.g., wherein the capture probes are from a cell or subject which express gene X or from a cell or subject in which a gene X-mediated response has been elicited, e.g., by contact of the cell with nucleic acid X or protein X, or administration to the cell or subject of a nucleic acid X or

protein X; providing a second two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality, and each address of the plurality having a unique capture probe, e.g., wherein the capture probes are from a cell or subject which does not express gene X (or does not express as highly as in the case of the cell or subject described above for the first array) or from a cell or subject which in which a gene X-mediated response has not been elicited (or has been elicited to a lesser extent than in the first sample); contacting the first and second arrays with one or more inquiry probes (which are preferably other than a nucleic acid X, protein X, or antibody specific for protein X), and thereby evaluating the plurality of capture probes. Binding, e.g., in the case of a nucleic acid, hybridization with a capture probe at an address of the plurality, is detected, e.g., by signal generated from a label attached to the nucleic acid, polypeptide, or antibody.

The invention also features a method of analyzing a plurality of probes or a sample. The method is useful, e.g., for analyzing gene expression. The method includes: providing a first two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality having a unique capture probe, contacting the array with a first sample from a cell or subject which express or mis-express gene X or from a cell or subject in which a gene X-mediated response has been elicited, e.g., by contact of the cell with nucleic acid X or protein X, or administration to the cell or subject of nucleic acid X or protein X; providing a second two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality, and each address of the plurality having a unique capture probe, and contacting the array with a second sample from a cell or subject which does not express gene X (or does not express as highly as in the case of the as in the case of the cell or subject described for the first array) or from a cell or subject which in which a gene X-mediated response has not been elicited (or has been elicited to a lesser extent than in the first sample); and comparing the binding of the first sample with the binding of the second sample. Binding, e.g., in the case of a nucleic acid, hybridization with a capture probe at an address of the plurality, is detected, e.g., by a signal generated from a label attached to the nucleic acid, polypeptide, or antibody. The same array can be used for both samples or different arrays can be used. If different arrays are used the same plurality of addresses with capture probes should be present on both arrays.

In another aspect, the invention features a method of analyzing gene X, e.g., analyzing the structure, function, or relatedness to other nucleic acids or amino acid sequences. The method includes: providing a nucleic acid X or protein X amino acid sequence; comparing the nucleic acid or amino acid sequence with one or more sequences from a collection of sequences, e.g., a nucleic acid or protein sequence database; to thereby analyze gene X.

The following examples are meant to illustrate, not limit, the invention.

EXAMPLES

Example 1. Methods and Materials

Tissue samples and tissue microarrays (TMA)

All human tissue was collected following NIH guidelines and using protocols approved by the Institutional Review Boards of relevant institutions (see below).

Fresh tissue specimens obtained from the Brigham and Women's Hospital, Massachusetts General Hospital, and Faulkner Hospital (all Boston, MA), Duke University (Durham, NC), University Hospital Zagreb (Zagreb, Croatia), and the National Disease Research Interchange (Philadelphia, PA) were snap frozen on dry ice and stored at -80°C until use. Tumors with significant DCIS components were identified based on pathology reports and confirmed by microscopic examination of hematoxylin-eosin stained frozen sections. Of the tumors used for SAGE analysis, D1, D3, D4, D5 and D6 were high-grade, comedo DCIS, and D2, D7 and T18 were intermediate-grade DCIS with no necrosis. Tumors used for mRNA *in situ* hybridization and immunohistochemistry included DCIS tumors of all three (low, intermediate, and high grade) histologic types. Most of the tumors used for *in situ* hybridization and immunohistochemistry were DCIS with concurrent invasive carcinoma and pure DCIS (i.e., without concurrent invasive carcinoma), respectively. Tumors D3 and D6 used for SAGE were pure DCIS. The larger representation of frozen/fresh DCIS tumors with concurrent invasive disease was due to logistic issues; it is extremely difficult to obtain frozen or fresh pure DCIS specimens, especially ones with long term clinical follow up data. For *in situ* hybridization, 5 μm thick frozen sections were mounted on silylated slides (CEL Associates Inc, Pearland, TX), air dried, and stored at -80°C until use.

Tissue microarrays (TMAs) were: (1) obtained from commercial sources (Imgenex, San Diego, CA (49 invasive breast tumors); Ambion, Austin, TX (92 primary invasive tumors and 41 distant metastases)); (2) provided by the Cooperative Breast Cancer Tissue Resource, Rockville, MD (40 normal breast tissue samples, 10 pure DCIS tumors, 10 DCIS with concurrent invasive tumors, and 192 primary invasive breast tumors); (3) generated at Johns Hopkins University, Baltimore, MD (299 invasive breast tumors and 10 distant metastases) and at Beth Israel Deaconess Medical Center (30 invasive breast tumors and 70 pure DCIS tumors of different histologic grades, all with matched normal breast tissue) following published protocols [Kononen et al. (1998) Nat. Med. 4:844-847]. With the exception of the Imgenex and the DCIS arrays (1 mm punches), all TMAs contained 0.6 mm punches, with at least 2 punches/tumor in order to control for tumor and immunohistochemical staining heterogeneity.

Cell lines

Breast cancer cell lines were obtained from American Type Culture Collection (ATCC; Manassas, VA) or were generously provided by Drs. Steve Ethier (University of Michigan) and Arthur Pardee (Dana-Farber Cancer Institute). Cells were grown in media recommended by the provider.

Generation and analysis of SAGE libraries from normal and malignant breast tissue

SAGE libraries were generated from DCIS tumors and normal breast tissue and analyzed essentially as previously described as part of the National Cancer Institute Cancer Gene Anatomy Project [Porter et al. (2001) Cancer Res. 61:5697-5702; Krop et al. (2001) Proc. Natl. Acad. Sci. U.S.A 98:9796-9801; Lal et al. (1999) Cancer Res. 59:5403-5407; and Boon et al. (2002) Proc. Natl. Acad. Sci. U.S.A. 99:11287-11292]. Two of the DCIS tumors were pure DCIS (D3 and D6) and the others were obtained from patients with concurrent invasive breast carcinomas. Epithelial cells from normal breast tissue (N1 and N2) and some tumors (D2, D3, D6, and D7) were purified using epithelial cell-specific monoclonal antibody (BerEP4)-coated magnetic beads (Dynal, Oslo, Norway); other tumors were macroscopically dissected based on adjacent hematoxylin-eosin stained slides. Approximately 50,000 SAGE tags were obtained from each library. For further analyses libraries were normalized to the library with the highest tag number (89,541 total tags). Hierarchical clustering was applied to data using the Cluster

program developed by Eisen et al. [Eisen et al. (1998) 95:14863-14868]. Differentially expressed genes were identified based on statistical analysis of comparisons of groups of normal (2 samples), DCIS (8 samples), and invasive breast cancer (9 samples) SAGE libraries using the SAGE2000 software [Velculescu et al. (1995) Science 270:484-487]. Similarly for the identification of genes specifically expressed in DCIS or invasive breast cancer, the 8 DCIS samples were treated as a group and the 9 invasive or metastatic patients were treated as another group. First, the SAGE tag numbers highest in two normal libraries (N1 and N2) were used as the cut-off and tag numbers in the DCIS and invasive libraries above this "normal" value were calculated using a two-sided Fisher-exact test without multiple comparisons (see Table 4). In a second test, ROC (receiver operating characteristic) curve analysis was used to choose the "best" cut-off for values (Table 4). A ROC area of 0.50 is no better than chance and a ROC area of 1.00 is the best possible.

mRNA in situ hybridization

To generate templates for *in vitro* transcription reactions, 300-500 base pair fragments derived from the 3' untranslated region of the selected genes were PCR amplified and subcloned into the pZERO 1.0 expression vector (Invitrogen, Carlsbad, CA). pZERO 1.0 contains a multiple cloning site bounded by SP6 and T7 RNA polymerase promoters; therefore the same plasmid can be used for the generation of sense and anti-sense riboprobes for mRNA *in situ* hybridizations. Digitonin-labeled sense and anti-sense riboprobes were generated and mRNA *in situ* hybridization was performed as described [Qian et al. (2001) Genes Dev. 15:2533-2545; Porter et al. (2003a) Mol. Cancer Res. 1:362-375]. The hybridized sections were observed with a NIKON microscope, images were obtained using a SPOT CCD camera, and the images were processed with the Adobe (San Jose, CA) Photoshop program. Hybridizations were considered successful if the control sense probe gave no significant signal. The intensity and distribution of the hybridization signal were scored (0-3 for intensity and 0-3 for distribution using the scoring scheme described below for immunohistochemistry) independently by three investigators.

Immunohistochemistry

The expression of the indicated genes in primary breast tumors was determined by immunohistochemical analysis of eight tissue microarrays that contained evaluatable paraffin-

embedded specimens derived from 80 DCIS, 675 primary invasive breast cancer, and 33 distant metastases. Antigen Retrieval Citra solution (Research Genetics, San Ramon, CA) and boiling in a microwave oven (5 minutes at high power) were used to enhance staining. Isotype control serum was used for negative control samples. A standard indirect immunoperoxidase protocol with 3,3'-diaminobenzidine as chromogen was used for the visualization of antibody binding (ABC-Elite; Vector Laboratories, Burlingame, CA).

Primary antibodies used were as follows: mouse monoclonal antibody specific for human psoriasin ("anti-psoriasin") [Enerback et al. (2002) Cancer Res. 62:43-47]; affinity-purified rabbit polyclonal antibody specific for human Connective Tissue Growth Factor (CTGF) ("anti-CTGF") (a generous gift of Dr. D. Brigstock, Childrens' Research Institute, Colombus, OH); affinity-purified rabbit polyclonal antibody specific for human Trefoil Factor 3 (TFF3) ("anti-TFF3") (a kind gift of Prof. Hoffman, Universitaetsklinikum, Magdeburg, Germany); mouse monoclonal antibodies specific for human interleukin-8 (IL-8) ("anti-IL-8"), GRO-1 ("anti-GRO-1"), and GRO-2 ("anti-GRO-2") (R&D Systems, Minneapolis, MN); monoclonal antibody specific for human osteonectin (SPARC) ("anti-SPARC") (Hematologic Technologies, Essex Junction, VT); and monoclonal antibody specific for human fatty acid synthase (FASN) ("anti-FASN") (Transduction Labs. San Diego, CA). Mouse monoclonal antibodies specific for interleukin-1β (IL1β) and CCL3 (chemokine (CC motif) ligand 3, also known as macrophage inhibitory protein 1α (MIP1α)) were purchased from R&D (Minneapolis, MN) while anti-CD45 mouse monoclonal antibody was obtained from DAKO (Carpinteria, CA). Antibodies were used at a 1:100 dilution in PBS (phosphate buffered saline) containing 10% heat-inactivated goat serum.

Antibody staining was subjectively scored by three investigators independently on a scale of 0-3 for intensity (0=no staining, 1=faint signal, 2=moderate and 3=intense staining) and 0-3 for extent (0=no, 1=≤30%, 2=30-70%, and 3= ≥70% positive cells) of staining. Cumulative scores were obtained by adding the average intensity and extent scores assigned by the three independent observers. For statistical analyses a cumulative score at or above 3 was considered positive. Relationships between the expression of genes determined by mRNA *in situ* hybridization or immunohistochemistry were analyzed by Fishers exact test without correction for multiple comparisons.

Statistical analyses of clinical correlates

The relationship of gene expression to clinico-pathologic parameters and the association between the expression of different genes determined by immunohistochemistry were analyzed by the following statistical methods.

The eight individual tissue microarray datasets and a combined dataset were analyzed for association of gene expression positivity and prognostic factors using a logistic regression model (with gene expression positivity as the outcome), and a forward, or step-up, selection procedure to determine the best fitting model. Clinico-pathologic factors analyzed were: expression of the estrogen and progesterone receptors and HER2 by immunohistochemistry, histologic grade, TNM (tumor, node metastasis) stage, tumor size, number of positive lymph nodes, patient age, and overall and distant metastasis-free survival. If all patients or no patients with a particular level of a covariate demonstrated gene expression positivity, then the logistic regression did not converge and a significance level was obtained using Fisher's exact test. If, however, there remained some patients with and without gene expression positivity after deleting patients with the particular level of the covariate, then a step-up logistic regression was performed on them. The significance of the variables in the logistic regression models was tested using likelihood ratio tests. The cut-off used for entry into the model was α=0.05. In addition to the analyses described above, Kaplan-Meier curves were generated and Cox models were run for two datasets that contained survival information. Calculated times to distant failure and times to survival were used and were based on the failure/death and accession dates.

Generation of SAGE libraries from epithelial and non-epithelial cells of normal breast and DCIS tissue

The procedure described in this section was used to obtain the data described in Example 6.

Some of the cell types present in normal and cancerous breast tissue comprise a minor fraction (a few percent) of all cells of the relevant tissue; thus, genes that are specifically expressed in such cell types may not be detected by analysis of the whole tissue. In order to analyze the comprehensive gene expression profiles of purified luminal epithelial cells, myoepithelial cells, endothelial cells, fibroblasts and leukocytes isolated from normal breast tissue and breast carcinomas using SAGE, a purification procedure that allows the isolation of pure cell populations was developed. A brief outline of the procedure is depicted in Fig. 1. In

order to isolate specific cell types, antibodies specific for cell type-specific cell surface markers and magnetic beads were employed using well-established methods. Thus, luminal mammary epithelial cells were isolated using the BerEp4 monoclonal antibody, myoepithelial cells with a monoclonal antibody specific for CD10/Calla, infiltrating leukocytes with a monoclonal antibody specific for the CD45 panleukocyte marker, and endothelial cells with the P1H12 monoclonal antibody that binds to an endothelial-specific cell surface protein. Essentially all the cells separated as luminal cells from breast cancer samples would be breast cancer cells. Thus, as used herein, breast "stromal cells" are breast cells other than epithelial cells. No antibody specific for a cell surface marker specific for fibroblasts was identified. Therefore, on the assumption that after removal of the above listed cell types the "leftover" cells were enriched for fibroblasts, the leftover cells were considered to be a "fibroblast enriched" fraction. The success of the purification procedure and the purity of each cell fraction were confirmed by a RT-PCR (reverse transcription-polymerase chain reaction) analysis of RNA isolated from 1/10 of the cells using the cell type specific marker used for the isolation of the cells. In Fig. 2 is shown the results of such an RT-PCR analysis of RNA isolated from: (a) luminal epithelial cells ("epithelium"), myoepithelial cells ("myoepithelium"), leukocytes, and endothelial cells ("endothelium") purified as described above from two DCIS tumors (DCIS6 and DCIS7); and (b) leukocytes and endothelial cells ("endothelium") from normal breast tissue . The PCR phases of the RT-PCRs were carried out with oligonucleotide primers specific for β-actin ("BAC") and L19 (both constitutively expressed by all cells), HER2 (expressed by some breast cancers), CALLA (a myoepithelial cell marker), CD45 (a pan-leukocyte marker), and an endothelial cell surface protein ("CDH5"; an endothelial cell marker). PCR were performed for 25, 30, and 35 cycles.

The cells not used for the RT-PCR analysis were used for the generation of micro-SAGE libraries. SAGE libraries were generated from luminal epithelial cells, myoepithelial cells, infiltrating lymphocytes, and endothelial cells from a normal breast reduction tissue (1 library/cell type) and from DCIS luminal and myoepithelial cells, infiltrating lymphocytes and endothelial cells (2 different tumors-2 libraries/cell type). Approximately 50,000 SAGE tags were obtained from each library, thereby enabling the analysis of thousands of unique transcripts. Based on these SAGE data, genes that are differentially expressed in specific cell types of normal and DCIS breast tissue were identified.

Ligand binding, cell growth, migration and invasion assays

N-terminal or C-terminal alkaline phosphatase (AP) CXCL14 fusion proteins were generated using the AP-TAG-5 expression vector (GenHunter, Nashville, TN). Mammalian cells were transfected with Fugene6 (Roche, Indianapolis, IN), Lipofectamine or Lipofectamine 2000 (LifeTechnologies, Rockville, MD) reagents. *In vivo* and *in vitro* ligand binding assays were carried out on primary tissues and cell lines using AP-CXCL14 essentially as described (Flanagan et al (1990) Cell 63:185-194; Porter et al. (2003b) Proc. Natl. Acad. Sci. USA 100:10931-10936]. Briefly, frozen sections of various human specimens were fixed, incubated with either AP-CXCL14 fusion protein or AP control conditioned medium, rinsed, and then incubated with AP substrate forming a blue/purple precipitate. For *in vitro* assays cells in suspension with conditioned media containing either AP alone or AP-CXCL14 fusion protein, rinsed, and then assayed for bound AP activity.

To determine the effect of CXCL14 on cell growth, MDA-MB-231 and MCF10A cells were plated (4,000 cells/well) in a 24 well tissue culture plate and grown in conditioned medium containing AP or AP-CXCL14. Conditioned medium was generated by transfecting 293 cells with pAP-tag5 or pAP-CXCL14 plasmids and growing them in McCoy's medium supplemented with 10% fetal bovine serum (FBS) (used for MDA-MB-231 cells) or in MCF10A media (ATCC; used for MCF10A cells). Cells were counted (3 wells/time point) on days 1, 2, 4, 6, and 8 after plating. 10 nM CXCL12 was used as a positive control in the experiment with MDA-MB-231 cells. The experiments were repeated three times.

In order to determine if CXCL14 binding to breast cancer cells has an effect on cell migration and invasion, the ability of conditioned medium containing AP-CXCL14 or pCDNA3.1 expressing HA (hemagglutinin)-tagged CXCL14 to induce the migration and invasion of MDA-MB-231 cells was tested using BIOCOAT Matrigel invasion chambers essentially as previously described [Muller (2001) Nature 410:50-56]. For invasion assays, cells were plated at a concentration of $2.5x10^4$ cells/well and assayed 24 hours later. For migration assays cells at a concentration of $1.25x10^4$ cells/well were used and cell numbers were determined 12 hours later. Conditioned media from cells transfected with pAP-Tag5 or pCDNA 3.1 empty vectors were used as negative controls.

Example 2. Normal and Cancerous Breast Transcriptomes Determined by SAGE

Genes differentially expressed between normal and cancerous breast tissues were identified using SAGE. Confirming previous studies of the inventors using a smaller number of SAGE libraries [Porter et al. (2001) Cancer Res. 61:5697-5702], the most dramatic difference in gene expression patterns was found to occur at the normal to *in situ* carcinoma transition and involves the uniform down-regulation of 32 genes (Table 1); while 34 tags and their corresponding genes are shown in Table 1, two genes (encoding interleukin-8 and GRO10 were each represented by two tags. Table 1 shows data from two normal breast tissue samples (N1 and N2), eight DCIS samples (D1-D7 and T18), six invasive breast cancer samples (I1-I6), two lymph node metastases (LN1 and LN2) from the same subjects that samples I1 and I2 were obtained from, and a lung metastasis (MET) from a breast cancer patient. In Table 1 and subsequent tables, Unigene identification numbers for relevant genes are shown in columns labeled "Unigene". The contents (e.g.., nucleic acid sequences and amino acid sequences) of database submissions identified by all the listed Unigene identification numbers are incorporated herein by reference in their entirety. Since many of the genes whose expression was found to be down-regulated after the normal to *in situ* transition encode secreted proteins and genes related to epithelial cell differentiation, loss of the differentiated epithelial phenotype and abnormal autocrine/paracrine interactions appear to play an essential role in the initiation of breast tumorigenesis.

The inventors also identified 144 genes up-regulated in a fraction of *in situ,* invasive and metastatic tumors (Table 2). The normal, DCIS, and lymph node samples studied in this analysis were the same as those shown in Table 1. Invasive breast cancer samples I1-I5 were the same as samples I1-I5 shown in Table 1 and T15 was an additional invasive breast cancer sample. Nearly 1/4 of the relevant SAGE tags currently have no database match indicating that many transcripts specifically expressed in certain breast carcinomas remain to be identified.

Table 1. Genes universally down-regulated in breast cancer irrespective of pathologic stage

SEQ ID NO:	Tag sequence	Unigene	Gene	N1	N2	D1	D2	D3	D4	D5	D6	D7	T18	I1	I2	I3	I4	I5	I6	LN1	LN2	MET
	Secreted proteins																					
1	AAATATCCAG	624	interleukin 8*	15	5	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
2	TGGAAGCACT	624	interleukin 8*	368	352	8	39	12	1	0	94	15	0	2	0	1	0	0	0	0	0	0
3	AAGCTCGCCG	62492	secretoglobin, family 3A, member 1 (HIN-1)	125	44	0	0	0	3	0	9	0	0	0	0	0	0	0	0	0	0	4
4	TTGAAACTTT	789	CXCL1 (GRO1) *	394	453	11	12	14	1	0	61	1	4	0	0	1	0	1	0	0	0	2
5	TTGCAGGCTC	789	CXCL1 (GRO1) *	13	40	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
6	ATAATAAAAG	89690	GRO3	24	205	4	0	6	4	4	2	0	5	7	5	3	8	4	8	6	7	11
7	TTGGTTTTTG	164021	small inducible cytokine subfamily B (Cys-X-Cys), member 6	56	16	0	3	0	0	0	1	0	0	0	0	1	0	0	0	0	0	4
8	GAGGGTTTAG	75498	small inducible cytokine subfamily A (Cys-Cys), member 20	44	30	2	0	0	0	0	2	2	0	0	0	1	0	0	0	0	0	0
9	GTACTAGTGT	303649	small inducible cytokine A2	33	12	2	0	3	1	0	2	1	0	2	3	3	0	1	4	0	0	2
10	GCCTTAACAA	239138	pre-B-cell colony-enhancing factor	45	30	11	15	0	7	6	17	9	2	7	4	5	4	1	4	4	3	7
11	GCCTTGGGTG	2250	leukemia inhibitory factor	64	135	0	3	8	1	0	4	10	0	0	0	1	0	0	4	0	0	0
	Cell surface proteins/receptors																					
12	ACCAAATTAA	51233	tumor necrosis factor receptor superfamily, member 10b	31	35	11	0	0	1	2	6	13	2	4	8	1	3	7	12	6	7	7
13	AGAAAGATGT	78225	annexin A1	83	77	11	3	15	12	10	9	4	23	4	16	19	3	7	16	6	0	20
14	TGACTGGCAG	278573	CD59 antigen p18-20	49	33	15	9	11	0	4	6	9	4	4	1	14	11	1	0	0	3	5
15	GTCCGAGTGC	374348	ESTs, Highly similar to A42926 L6 surface protein	134	96	11	33	11	1	2	23	13	4	2	0	0	8	0	8	2	3	5
	Cell growth and survival																					
16	GCTTGCAAAA	372783	superoxide dismutase 2, mitochondrial	210	121	6	12	5	3	0	10	3	0	4	0	1	1	1	4	6	3	7
17	ACCAGGCCAC	101382	tumor necrosis factor, alpha-induced protein 2	24	23	0	0	0	9	0	7	7	0	0	1	1	0	10	0	2	0	4
18	TTTGAAATGA	28491	spermidine/spermine N1-acetyltransferase	129	133	13	45	37	29	6	20	55	5	4	12	40	11	13	20	4	4	7
19	CTTGCAAACC	127799	baculoviral IAP repeat-containing 3	16	26	0	6	2	1	0	1	2	0	2	1	1	0	1	4	0	1	4
20	CCATTGAAAC	75517	laminin, beta 3	20	21	2	3	2	1	0	2	0	7	0	0	5	1	1	0	0	1	2
21	CCCGAGGCAG	155223	stanniocalcin 2	62	23	4	6	0	0	2	4	4	2	0	4	6	3	4	0	0	1	2
22	CTGGCCCTCG	348024	v-ral simian leukemia viral oncogene homolog B	296	145	55	117	9	0	31	12	74	69	2	1	0	0	1	0	2	3	2
23	GACACGAACA	25829	RAS, dexamethasone-induced 1	45	30	6	0	8	4	0	2	2	9	9	3	1	7	0	0	2	4	11
24	GCTGCCCTTG	272897	tubulin, alpha 3	103	75	13	30	3	10	8	18	32	2	11	9	13	15	12	20	6	12	16
	Differentiation																					
25	CGAATGTCCT	335952	keratin 6B	53	49	0	0	17	0	0	4	0	0	0	0	0	1	0	0	0	0	2
26	CTCACTTTTT	76722	CCAAT/enhancer binding protein (C/EBP), delta	154	112	38	45	11	16	33	22	22	12	7	4	12	17	0	0	4	6	23
	Unknown function																					
27	AGAATTTAGG	105094	ESTs	13	26	2	0	0	0	0	0	0	2	0	1	3	0	1	0	2	0	0
28	AGTCAAAAAT	NA	No reliable match	13	14	0	0	0	0	0	1	4	0	0	0	0	0	1	0	0	0	0
29	ATTAGTGTTG	23740	KIAA1598 protein	15	7	0	0	0	0	0	1	1	0	0	0	1	0	0	0	4	0	0
30	CTTTGGAAAT	6820	Homo sapiens cDNA FLJ32718 fis	16	54	4	0	3	1	0	4	5	0	0	0	0	0	0	8	2	0	9
31	GCAACTTAGA	NA	No reliable match	29	21	6	3	0	1	0	2	1	7	0	0	4	3	0	0	0	0	0
32	GGGACGAGTG	NA	No reliable match	250	460	48	493	34	29	53	89	51	49	25	9	8	117	3	32	16	19	88
33	GGGTTTGTTT	75969	proline rich 2	38	44	4	0	3	4	4	20	8	0	2	1	6	11	1	8	2	1	14
34	GTCTTAAAGT	177781	Homo sapiens, clone IMAGE:4711494, mRNA	100	58	0	0	3	1	0	21	8	0	2	0	5	4	1	8	4	1	2

*From interleukin 8 and GRO1 two independent SAGE tags were derived and both were down-regulated in tumors.

Table 2. Genes up-regulated in breast cancer

Tag	Unigene	Gene	Normal			In situ									Invasive							Metastatic			
			N1	N2	Ave	D1	D2	D3	D4	D5	D6	D7	T18	Ave	I1	I2	I3	I4	I5	T15	Ave	LN1	LN2	MET	Ave
Secreted proteins and ECM related																									
ATGTCTTTTC	1516	insulin-like growth factor binding protein 4	4	5	4	17	36	6	32	59	9	9	4	21	13	29	33	7	19	24	21	8	29	2	13
CATATCATTA	119206	insulin-like growth factor binding protein 7	0	0	0	11	6	6	63	39	4	3	42	22	49	63	59	59	28	80	57	55	12	18	28
CTCCACCCGA	352107	trefoil factor 3 (intestinal)	34	7	21	511	854	17	26	451	31	38	261	274	369	124	15	0	94	16	103	285	244	2	177
ACGTTAAAGA	350570	dermcidin (IBC-1)	0	0	0	0	0	0	1	0	0	0	0	0	177	101	3	0	0	12	49	199	0	0	66
ATTTTCTAAA	91011	anterior gradient 2 homolog	4	7	5	13	75	2	39	2	7	5	0	18	13	17	3	0	12	0	7	2	54	0	19
AGTGGTGGCT	230	fibromodulin	0	0	0	17	0	2	22	0	0	2	34	9	34	36	3	1	70	12	26	22	6	25	18
ATCTTGTTAC	287820	fibronectin 1	0	0	0	4	0	5	7	14	0	2	2	4	2	4	15	4	21	12	10	2	1	0	1
TTATGTTTAA	79914	lumican	0	0	0	2	3	2	28	4	1	1	11	6	0	20	21	1	25	20	14	16	6	11	11
CTCATCTGCT	82109	syndecan 1	0	0	0	0	3	2	25	14	20	2	11	9	4	5	10	36	10	0	11	10	1	9	7
ACATTCCAAG	245188	tissue inhibitor of metalloproteinase 3	0	2	1	13	24	0	12	12	2	7	9	10	7	3	9	1	15	4	6	6	9	7	7
CCAGAGAGTG	180884	carboxypeptidase B1 (tissue)	0	0	0	0	9	0	0	0	0	21	0	4	107	115	0	1	0	0	37	0	354	2	119
TTTGGTTTTC	179573	collagen, type I, alpha 2	0	0	0	231	0	8	175	53	4	3	12	61	92	90	159	11	158	40	92	138	70	48	85
ACCAAAAACC	172928	collagen, type I, alpha 1	2	5	3	282	3	8	108	41	22	8	85	70	92	71	83	3	185	189	104	153	34	57	81
TGCAAATGAC	172928	collagen, type I, alpha 1	2	2	2	191	0	8	260	80	9	0	11	70	184	91	218	23	254	40	135	252	87	39	126
TTTGTTTTTA	3622	procollagen-proline, 2-oxoglutarate 4-dioxygenase	0	0	0	0	3	2	3	2	1	4	2	2	7	7	27	4	21	4	11	2	18	0	7
TGGCCCCAGG	268571	apolipoprotein C-I	2	2	2	8	0	3	44	47	1	3	19	16	87	58	22	8	45	92	52	81	28	32	47
CGACCCCACG	169401	apolipoprotein E	5	2	4	13	0	15	16	33	4	2	65	18	29	37	14	3	54	173	52	31	28	32	31
AACACAGCCT	170250	complement component 4A	5	5	5	25	3	0	52	4	1	5	110	25	29	17	51	0	160	84	57	4	46	7	19
GAATTTCCCA	2253	complement component 2	0	0	0	17	0	0	1	2	0	0	19	5	2	7	1	6	1	8	4	6	1	7	5
CAAACTAACC	153261	immunoglobulin heavy constant mu	0	0	0	11	0	2	50	0	1	0	28	11	172	70	40	1	0	0	47	320	13	193	176
GAAATAAAGC	300697	immunoglobulin heavy constant gamma 3	0	0	0	55	0	129	459	10	1	0	247	113	721	665	53	43	0	2442	654	1445	109	770	775
AAACCCCAAT	181125	immunoglobulin lambda joining 3	0	0	0	15	0	17	102	4	1	1	44	23	163	87	78	3	0	241	95	258	10	38	102
Cell surface proteins/receptors																									
AAGCACAAAA	9963	TYRO protein tyrosine kinase binding protein	0	0	0	2	0	0	13	12	0	0	0	3	20	12	8	3	16	12	12	14	7	23	15
TGGTTTGCGT	6459	putative G-protein coupled receptor GPCR41	4	7	5	29	36	5	36	45	13	23	12	25	27	25	5	72	12	8	25	24	37	16	25
TACAATAAAC	9071	progesterone receptor membrane component 2	0	0	0	4	9	0	17	18	1	5	0	7	9	5	14	6	18	8	10	20	16	9	15
AGGAAGGAAC	323910	v-erb-b2	0	0	0	8	9	11	157	43	110	24	81	55	60	42	13	11	6	96	38	104	12	4	40
ACATTCTTTT	82226	glycoprotein (transmembrane) nmb	2	0	1	4	0	2	7	8	1	0	5	3	4	9	13	18	9	36	15	10	6	25	14
CACCCTGTAC	25450	solute carrier family 29	0	0	0	0	0	2	3	8	0	0	44	7	4	1	5	157	9	20	33	2	9	4	5
GTTCACATTA	84298	CD74 antigen	7	33	20	29	6	25	188	70	6	13	28	46	159	208	226	32	428	474	254	203	72	72	115
CAAGCAGGAC	179516	integral type I protein	2	0	1	17	15	0	38	6	2	4	64	18	29	15	12	30	13	44	24	14	28	16	19
TGCTGCCTGT	118110	bone marrow stromal cell antigen 2	4	9	6	13	57	2	38	14	12	85	57	35	22	41	22	10	21	153	45	6	78	41	42
CCCATCATCC	306122	glycoprotein, synaptic 2	0	0	0	0	6	0	7	16	1	10	16	7	4	8	17	1	15	4	8	2	6	7	5
GCAGTGGCCT	184276	solute carrier family 9	5	7	6	19	96	8	13	53	13	25	9	30	45	32	6	7	19	12	20	31	32	13	25
Cell cycle and apotosis																									
AAAGTCTAGA	82932	cyclin D1	7	2	5	19	63	6	42	39	29	17	4	27	56	114	36	3	53	12	46	20	140	2	54
CTGGCGCCGA	183180	APC11 anaphase promoting complex subunit 11	4	2	3	11	42	2	7	29	2	2	12	13	22	17	19	11	15	28	19	26	28	20	24
Protein synthesis, transport and degradation																									
TTTCAGAGAG	75975	signal recognition particle 9kDa	13	9	11	86	18	23	92	64	10	34	25	44	51	71	83	48	89	24	61	53	60	41	51
TTCTTGCTTA	169895	ubiquitin-conjugating enzyme E2L 6	0	0	0	0	6	3	7	12	2	7	11	6	9	12	14	6	6	36	14	4	25	5	11
GAGAGTGGGG	252259	ribosomal protein S3	0	0	0	6	0	0	0	0	0	0	14	3	18	4	0	0	0	12	6	10	25	0	12
Transcription, chromatin, other nuclear proteins																									
TGAGCAAGCC	27801	zinc finger protein 278	0	0	0	6	0	2	1	2	1	0	7	2	18	11	3	0	9	4	7	14	16	2	11
CCTGTACCCC	32317	high-mobility group 20B	0	0	0	2	3	3	3	8	4	6	25	7	7	7	8	7	6	12	8	2	7	0	3
CCTTTCACAC	278589	general transcription factor II, i	4	2	3	13	15	5	22	59	1	13	14	18	27	24	31	47	37	8	29	16	35	9	20
CACCAGCATT	75847	CREBBP/EP300 inhibitory protein 1	4	0	2	19	15	3	22	18	0	7	30	14	27	15	15	0	9	0	11	22	21	2	15
TTTTGTAATT	75890	membrane-bound transcription factor protease	0	0	0	0	3	3	4	0	1	3	14	4	4	9	8	0	7	4	5	2	16	9	9
GTGCAGGGAG	79414	prostate epithelium-specific Ets transcription factor	2	0	1	8	21	0	57	33	11	13	110	32	56	54	28	3	32	24	33	59	41	2	34
ATGACTCAAG	239752	nuclear receptor subfamily 2	0	0	0	15	9	3	19	39	7	16	5	14	27	21	24	29	23	8	22	18	48	11	26
ATTGTTTATG	181163	high-mobility group nucleosomal binding domain 2	2	9	6	13	18	3	55	55	4	21	14	23	60	53	60	43	47	20	47	51	34	9	31
AAGGATGCCA	169946	GATA binding protein 3	4	0	2	55	9	0	1	14	9	24	9	15	13	7	17	0	26	16	13	8	38	0	15
CTTGTAATCC	183253	nucleolar RNA-associated protein	9	2	6	4	72	78	22	55	7	80	4	40	27	21	14	19	7	104	32	4	62	7	24
TAGTTTGTGG	78934	mutS homolog 2	0	0	0	8	9	5	4	8	0	0	4	5	13	12	12	15	4	0	9	37	10	11	19
Signal transduction																									
CGGTCTTATG	75842	dual-specificity phosphorylation regulated kinase 1A	0	0	0	2	0	0	15	27	4	0	5	7	7	11	18	21	7	8	12	4	3	2	3
TGAAAAGCTT	2384	tumor protein D52	2	2	2	19	21	5	26	47	5	15	2	17	49	44	22	69	19	28	38	18	109	25	50
TTAAGAGGGA	178137	transducer of ERBB2, 1	0	0	0	11	3	8	13	16	0	1	2	7	18	19	28	47	12	4	21	29	12	2	14
TATTTCACCG	138860	Rho GTPase activating protein 1	2	0	1	2	6	3	25	20	5	1	5	8	27	22	12	8	15	0	14	20	9	11	13
GTCTTTCTTG	151536	RAB13, member RAS oncogene family	2	2	2	13	0	2	12	20	0	6	4	7	11	19	32	37	25	8	22	22	9	13	14
CCAGGGGAGA	278613	interferon, alpha-inducible protein 27	0	0	0	4	36	3	4	90	5	176	2	40	0	21	5	1	3	104	23	2	31	77	37
GAGCAGCGCC	112408	S100 calcium binding protein A7 (psoriasin 1)	18	0	9	1018	3	3	373	16	1	2	890	288	0	0	0	1	0	20	4	0	0	0	0
GCTCTGCTTG	112408	S100 calcium binding protein A7 (psoriasin 1)	2	0	1	76	0	0	20	0	0	0	55	19	0	0	0	0	0	0	0	0	0	0	0
CGCCGACGAT	265827	interferon, alpha-inducible protein (IFI-6-16)	4	0	2	17	644	3	90	418	18	366	4	195	130	171	5	63	12	161	90	14	526	181	240
GTGTGTTTGT	118787	transforming growth factor, beta-induced, 68kD	0	0	0	8	0	2	10	6	1	0	4	4	13	11	21	8	22	44	20	24	10	9	14
CCAATAAAGT	101850	retinol binding protein 1, cellular	2	0	1	0	3	0	0	2	6	11	7	4	49	28	6	8	0	0	15	102	32	21	52
GTCTAGAATC	92384	vitamin A responsive; cytoskeleton related	0	0	0	21	6	0	25	6	1	4	32	13	16	7	21	11	15	24	15	20	10	5	12
ATCCGCGAGG	180142	calmodulin-like skin protein	0	0	0	0	0	3	22	0	20	0	0	6	47	25	0	52	19	0	24	20	0	0	7
GATTTTGCAC	274479	nucleoside diphosphate kinase 7	0	0	0	19	6	0	7	0	6	1	16	7	9	1	4	1	6	0	4	2	18	2	7

***The above sequences are SEQ ID NOs:35-97, respectively**

Table 2. *continued*

Tag	Unigene	Gene	Normal			In situ								Invasive							Metastatic			
			N1	N2	Ave	D1	D2	D3	D4	D5	D6	D7	T18	Ave	I1	I2	I3	I4	I5	T15	Ave	LN1	LN2	MET	Ave
Metabolism																									
ACCTTGTGCC	878	sorbitol dehydrogenase	0	2	1	4	18	0	20	4	1	3	9	7	22	26	1	6	110	4	28	4	95	0	33
TGCCGTTTTG	2006	glutathione S-transferase M3 (brain)	0	2	1	0	48	0	1	20	7	25	2	13	9	12	3	4	19	8	9	4	13	7	8
CCGTGCTCAT	9857	dicarbonyl/L-xylulose reductase	11	7	9	2	51	8	20	18	4	5	67	22	99	56	21	7	12	56	42	77	34	7	39
GTTTCTATCA	12540	lysophospholipase I	0	2	1	6	15	0	25	49	1	7	0	13	25	12	26	45	19	8	22	12	38	2	17
CAAATAAAAT	71465	squalene epoxidase	2	2	2	0	24	2	19	55	4	0	5	14	9	8	3	40	13	12	14	4	6	39	16
GGAACTTTTA	43857	similar to glucosamine-6-sulfatases	0	2	1	17	36	3	7	6	4	14	25	14	9	8	26	0	60	0	17	10	10	5	8
TTACCTTTTT	79222	galactosidase, beta 1	0	0	0	4	3	0	10	14	0	2	2	4	2	4	8	18	6	16	9	18	3	5	9
TTGGGGAAAC	81029	biliverdin reductase A	4	5	4	4	24	0	22	27	1	9	7	12	43	19	8	3	18	32	20	22	29	11	21
TGATCTCCAA	83190	fatty acid synthase	16	5	10	53	63	6	201	182	31	47	5	74	168	33	105	17	314	4	107	254	46	21	107
TTTGGTGTTT	83190	fatty acid synthase	5	0	3	8	24	2	57	27	5	28	21	21	36	41	62	14	57	12	37	28	10	4	14
TTAACCCCTC	78224	ribonuclease, RNase A family, 1 (pancreatic)	2	0	1	25	0	6	20	10	1	1	5	9	31	57	13	6	0	32	23	18	46	9	24
GCTTTGATGA	89649	epoxide hydrolase 1, microsomal (xenobiotic)	0	2	1	0	6	2	52	20	2	9	12	13	16	29	13	6	29	40	22	29	6	14	17
TACAGTATGT	170171	glutamate-ammonia ligase	0	5	2	13	12	3	36	82	4	24	228	50	4	19	87	26	56	56	41	4	16	0	7
TGGGGTTCTT	272499	dehydrogenase/reductase (SDR family) member 2	2	2	2	0	0	2	0	113	0	84	0	25	7	13	10	0	0	0	5	0	32	0	11
TTACTTCCCC	184641	fatty acid desaturase 2	2	0	1	2	0	0	138	29	9	2	0	22	29	19	10	32	43	4	23	53	4	4	20
AAGAATCTGA	183435	NADH dehydrogenase	0	0	0	15	0	3	31	31	1	3	0	10	34	20	14	17	35	0	20	71	46	2	39
GTCCCTGCCT	279837	glutathione S-transferase M2	0	5	2	4	18	0	10	53	1	6	5	12	4	13	22	8	47	0	16	4	12	11	9
AATATGTGGG	351875	cytochrome c oxidase subunit VIc	11	5	8	38	707	6	19	219	2	112	23	141	325	337	77	30	185	24	163	28	1250	14	431
GGAGCTCTGT	227750	NADH dehydrogenase 1 beta subcomplex, 4	4	5	4	11	39	5	17	27	5	21	14	17	18	11	30	22	29	16	21	16	31	9	19
GAAGGAGATA	171889	choline phosphotransferase 1	0	0	0	4	3	0	0	10	0	1	0	2	9	15	14	34	4	4	13	2	23	2	9
TCAGACTTTT	334305	diacylglycerol O-acyltransferase homolog 2	0	0	0	11	0	0	15	0	2	0	28	7	2	22	1	17	0	4	8	2	0	30	11
TCTTGTAACT	256549	nucleotide binding protein 2	0	0	0	0	12	0	9	4	5	4	2		11	13	4	1	4	48	14	22	12	2	12
ESTs																									
TGATGAGTGT	356209	ESTs	0	0	0	2	0	0	1	6	0	3	0	2	2	0	6	6	7	0	4	2	0	0	1
CTGCAACCTA	374393	ESTs	2	0	1	11	6	2	13	8	4	8	9	7	2	7	8	4	7	12	7	12	16	16	15
TGAGTGGTTT	29672	ESTs	0	0	0	4	0	0	3	14	0	0	2	3	4	3	10	12	6	8	7	2	6	5	4
CACTGTGTTG	350475	EST clone IMAGE:4430514	4	0	2	2	3	0	4	2	1	3	18	4	9	7	12	12	7	12	10	6	21	5	11
TTAAGAAGTT	275360	ESTs	7	0	4	15	0	3	63	0	0	0	2	10	2	1	55	0	18	0	13	14	6	0	7
GCGACAGTAA	170853	ESTs	0	0	0	4	0	0	6	16	0	5	16	6	9	8	9	3	15	20	11	2	1	4	2
TCAACTTGAA	99244	ESTs	0	0	0	21	3	3	7	4	12	0	0	6	16	19	9	3	10	0	9	28	40	16	28
TTTCTGGAGG	129943	KIAA0545 protein	2	0	1	15	3	3	4	12	6	1	2	6	16	12	12	6	7	4	9	20	6	13	13
GGGGCTGGAG	301685	KIAA0620 protein	0	0	0	11	6	5	13	29	6	6	4	10	2	9	14	6	7	16	9	8	13	18	13
GTCTCATTTC	90419	KIAA0882 protein	4	0	2	8	3	2	4	23	1	33	0	9	0	13	14	3	21	0	8	0	29	0	10
ACCGCCTGTG	79625	chromosome 20 open reading frame 149	2	5	3	4	36	2	1	80	4	121	19	33	4	7	13	19	21	12	13	6	6	9	7
GAAGAACAGA	29341	chromosome 20 open reading frame 81	0	0	0	13	3	3	4	16	0	2	2	5	4	9	14	8	6	0	7	6	15	7	9
TCGTAACGAG	11197	chromosome 20 open reading frame 92	4	2	3	11	0	0	15	8	4	3	23	8	25	8	18	19	4	12	14	22	10	16	16
GTGATGGGGC	62620	chromosome 6 open reading frame 1	2	0	1	2	12	0	13	2	0	4	11	5	16	3	6	6	13	0	7	20	10	9	13
GAGAGAAAAT	181444	hypothetical protein LOC51235	0	2	1	40	9	0	10	6	7	7	21	13	4	8	9	11	18	0	8	6	10	27	14
GCCCACATCC	84753	hypothetical protein FLJ12442	4	0	2	0	0	3	4	0	4	1	26	5	63	26	1	12	6	48	26	49	1	11	20
GTATTTAACT	209065	hypothetical protein FLJ14225	0	0	0	17	6	3	28	12	6	8	9	11	9	16	15	6	16	0	10	20	10	18	16
GGCTGGTCTC	324844	hypothetical protein IMAGE3455200	2	2	2	6	6	5	6	12	2	3	11	6	18	7	10	18	12	16	13	6	18	20	14
AACACTTCTC	333526	hypothetical protein MGC14832	4	0	2	2	6	0	25	8	1	2	4	6	27	19	4	0	9	4	10	18	6	4	9
AATAAAGAGA	28149	hypothetical protein BC010626	0	2	1	0	3	0	6	23	0	1	60	12	7	4	21	0	31	0	10	6	0	2	3
GAGAAACATT	267245	hypothetical protein FLJ14803	0	2	1	17	0	0	4	8	1	2	2	4	7	5	14	12	13	4	9	14	12	5	10
TTTGGTCTTT	109773	hypothetical protein FLJ20625	0	0	0	8	0	3	6	10	4	4	4	5	20	28	12	15	15	24	19	10	10	0	7
TGTGGTGGTG	83422	MLN51 protein	5	2	4	6	3	2	55	39	7	7	4	15	87	25	18	22	13	36	34	92	18	5	38
GAAAGATGCT	334370	brain expressed, X-linked 1	2	0	1	6	48	0	1	0	1	1	0	7	29	37	1	1	1	0	12	0	162	2	54
TAGCAGACCC	349196	myeloid/lymphoid or mixed-lineage leukemia	0	0	0	0	3	3	1	4	2	7	12	4	13	13	12	7	4	20	12	18	1	0	6

***The above sequences are SEQ ID NOs:98-144, respectively**

Table 2. *continued*

			Normal			In situ								Invasive							Metastatic			
Tag	Unigene	Gene	N1	N2	Ave	D1	D2	D3	D4	D5	D6	D7	T18	Ave	I1	I2	I3	I4	I5	T15	Ave	LN1	LN2	MET	Ave
No database match																									
AACGCTGCGA	NA	No reliable match	7	5	6	36	24	0	4	35	1	10	0	14	31	60	23	1	19	0	22	29	101	23	51
AATGGATGAA	NA	No reliable match	0	0	0	38	0	0	3	2	1	0	44	11	2	0	0	0	0	60	10	4	1	0	2
ACATCGTAGT	NA	No reliable match	0	0	0	0	15	0	3	31	0	2	2	7	13	20	4	4	10	4	9	0	60	0	20
ACCCGCCGGG	NA	No reliable match	11	7	9	103	18	3	4	0	1	6	166	38	20	8	0	1	4	193	38	31	23	0	18
AGTGCAGGGA	NA	No reliable match	0	0	0	2	0	2	15	2	0	0	37	7	38	9	23	1	1	48	20	26	0	7	11
ATCAAGAATC	NA	No reliable match	2	0	1	2	3	3	9	8	0	3	9	5	18	13	15	4	16	72	23	22	13	13	16
ATGTGGCACA	NA	No reliable match	4	2	3	2	24	0	20	31	1	9	34	15	18	16	12	44	23	8	20	14	15	9	12
CAAACCTTTA	NA	No reliable match	0	0	0	11	6	0	16	25	1	5	0	8	16	16	13	23	13	8	15	33	15	34	27
CAATGCTGCC	NA	No reliable match	11	12	11	53	12	3	23	33	9	3	64	25	580	145	18	18	26	44	139	588	28	11	209
CAGCTTAATT	NA	No reliable match	4	2	3	4	3	0	25	20	0	1	2	7	36	20	0	0	4	4	11	90	6	5	34
CCGACGGGCG	NA	No reliable match	4	2	3	67	3	0	3	0	1	4	87	21	7	0	0	0	0	181	31	4	7	0	4
CCTTTGAACA	NA	No reliable match	2	0	1	4	6	5	0	10	2	3	14	6	9	13	5	12	6	16	10	2	4	4	3
CCTTTGCCCT	NA	No reliable match	0	0	0	0	9	2	73	16	1	14	5	15	27	26	19	0	9	0	14	28	9	0	12
CGGTTTAATT	NA	No reliable match	2	0	1	23	0	0	12	10	1	3	53	13	13	9	26	3	25	16	15	20	0	0	7
CTTTATTCCA	NA	No reliable match	0	0	0	19	0	2	48	2	0	0	5	9	25	22	31	4	16	0	16	18	15	5	13
GAAGTCGGAA	NA	No reliable match	4	0	2	48	0	2	3	2	27	3	2	11	20	3	4	12	4	0	7	18	9	7	11
GATCTCGCAA	NA	No reliable match	4	7	5	44	21	0	31	25	7	1	0	16	40	13	12	22	16	4	18	47	38	64	50
GCACCTCCTA	NA	No reliable match	2	0	1	8	9	2	7	12	4	1	2	6	13	12	6	11	10	0	9	12	6	7	8
GCCGTGAGCA	NA	No reliable match	2	0	1	17	12	0	6	8	2	1	5	6	25	17	1	6	13	0	10	12	31	20	21
GGAAAGTGAC	NA	No reliable match	0	0	0	2	6	2	4	10	0	5	7	5	11	22	12	6	26	0	13	12	23	9	15
GGACCTTTAT	NA	No reliable match	2	0	1	23	3	0	1	23	1	0	37	11	2	1	1	0	1	0	1	4	3	0	2
GGCAGACAAT	NA	No reliable match	0	0	0	13	0	0	12	14	1	2	7	6	16	5	1	15	7	0	7	18	12	13	14
GGCAGCACAA	NA	No reliable match	0	5	2	23	18	0	16	27	20	12	5	15	49	11	5	12	6	4	15	35	25	29	30
GGTAGCTGCT	NA	No reliable match	0	0	0	6	3	0	3	20	0	6	14	7	7	4	4	4	3	0	4	2	1	4	2
GGTAGTTTTA	NA	No reliable match	13	0	6	59	21	3	32	41	2	13	18	24	18	28	39	0	59	16	26	18	79	0	32
GGTCAGTCGG	NA	No reliable match	5	5	5	76	15	2	0	0	39	3	102	30	25	3	1	7	1	80	20	18	13	2	11
GTAATCCTGC	NA	No reliable match	4	2	3	34	6	12	0	4	187	28	51	40	22	17	6	25	1	52	21	24	7	7	13
GTAGTTACTG	NA	No reliable match	2	2	2	8	120	0	1	25	0	21	4	22	38	33	13	7	19	0	18	8	172	4	61
TCACAGTGCC	NA	No reliable match	2	2	2	15	3	2	13	39	1	7	14	12	29	5	42	28	21	8	22	20	6	13	13
TCTGGTTTGT	NA	No reliable match	2	2	2	6	12	3	10	33	5	2	7	10	29	16	4	50	3	12	19	41	6	7	18
TGAAGCAGTA	NA	No reliable match	4	2	3	99	3	2	36	27	9	5	25	26	74	46	122	57	85	12	66	57	40	25	41
TGTCATAGTT	NA	No reliable match	0	0	0	0	15	0	9	55	0	3	9	11	34	42	9	4	34	4	21	6	197	0	68
TTACCATGAA	NA	No reliable match	2	0	1	0	6	0	3	18	1	1	0	4	51	41	4	1	7	0	18	73	9	2	28
TTCGGTTGGT	NA	No reliable match	2	0	1	101	3	0	55	16	0	0	7	23	58	40	40	1	60	4	34	55	22	11	29

Ave=average number of SAGE tags/histologic stage.

***The above sequences are SEQ ID NOs:145-178, respectively**

To identify overall similarities and differences among samples, the 19 SAGE libraries were analyzed by hierarchical clustering (Fig. 3A). A dendogram created using this program revealed that, while the two normal samples (N1 and N2) were more similar to each other than to any other samples, the primary invasive tumor and lymph node metastasis from the first patient (I1 and LN1) were more similar to each other than to any other sample and the primary invasive tumor and lymph node metastasis from the second patient (I2 and LN2) were more similar to each than to any other sample. *In situ* tumors, invasive tumors, and metastases did not form distinct clusters suggesting that none of these tumor classes is there a pronounced and common "*in situ*", "invasive", or "metastasis" signature. Correlating with this observation, clustering and other statistical analyses failed to identify any gene that was universally and specifically up or down-regulated in DCIS, invasive, or metastatic tumors (Fig. 3A). These findings confirm previous studies performed in invasive breast carcinomas and highlight the fact that DCIS tumors are just as heterogeneous at the molecular level as their invasive counterparts [Perou et al. (2000) Nature 406:747-752].

To analyze the relationships among DCIS tumors in more detail, hierarchical clustering was performed using the eight DCIS libraries (Fig. 3B). The expression profiles of 582 genes (Table 3) were included in this analysis; while 920 SAGE tags and their corresponding genes are listed in Table 3, many of the genes are represented by more than one tag. The program used for the clustering analysis (see Example 1) filtered for tags at least ten copies of which were present in at least one library and which were present in at least one library in a number at least ten-fold higher than in a library from another category of breast tissue. Genes expressed by non-epithelial cells apparently play a predominant role in defining the relatedness of samples since the BerEP4 purified (D2, D3, D6, and D7) and unpurified (D1, D4, D5, and T18) tumors formed two distinct clusters. Tumors also appeared to cluster according to their histologic grade with the high-grade tumors (D3, D6, D4, and D5) and the intermediate grade tumors (D2, D7) DCIS showing highest similarity to each other. However, T18, an intermediate grade, non-comedo DCIS, showed highest similarity to D1, a high grade comedo DCIS, suggesting that, despite its histologic features, this DCIS appears to have the molecular profile of a high grade, comedo DCIS.

Table 3. Genes employed for the clustering analysis shown in Fig. 3B

SEQ ID NO:	Tag	Unigene	Gene name
179	AGCGACAAAC	82109	syndecan 1
180	AGGAAGGAAC	323910	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)
181	CTGTTCCGGC	286192	dopamine and cAMP-regulated neuronal phosphoprotein 32
182	ATCGCTTTCT	177486	amyloid beta (A4) precursor protein (protease nexin-II, Alzheimer disease)
183	GTGGCCACGG	112405	S100 calcium binding protein A9 (calgranulin B)
184	ATGTGAAGAG	111779	secreted protein, acidic, cysteine-rich (osteonectin)
185	ATGTGAAGAG	126515	EST
186	TGAAGCAGTA	176626	hemogen
187	TGAAGCAGTA	326248	programmed cell death 4 (neoplastic transformation inhibitor)
188	ACCAAAAACC	172928	collagen, type I, alpha 1
189	TTTGCACCTT	75511	connective tissue growth factor
190	TTTGGTTTTC	21431	suppressor of fused homolog (Drosophila)
191	TTTGGTTTTC	179573	retinoblastoma binding protein 1
192	TGGAAATGAC	172928	collagen, type I, alpha 1
193	TGGAAATGAC	173648	ESTs, Weakly similar to zinc finger protein ZNF287 [Homo sapiens] [H.sapiens]
194	GGGCATCTCT	76807	major histocompatibility complex, class II, DR alpha
195	TTGCTGACTT	108885	collagen, type VI, alpha 1
196	TTGCTGACTT	238928	HT002 protein; hypertension-related calcium-regulated gene
197	TTTCAGAGAG	75975	signal recognition particle 9kD
198	TTTCAGAGAG	355743	ESTs, Highly similar to SR09 HUMAN Signal recognition particle 9 kDa protein (SRP9) [H.sapiens]
199	AACTGCTTCA	11538	actin related protein 2/3 complex, subunit 1B (41 kD)
200	ACTTACCTGC	12504	likely ortholog of mouse Arkadia
201	ACTTACCTGC	174031	cytochrome c oxidase subunit VIb
202	TGTGGTGGTG	83422	MLN51 protein
203	TGTGGTGGTG	223618	EST
204	TTACTTCCCC	184641	fatty acid desaturase 2
205	CATTTCAATA	75431	fibrinogen, gamma polypeptide
206	CATTTCAATA	32587	steroid receptor RNA activator 1
207	GTGCTGATTC	75584	polymyositis/scleroderma autoantigen 2 (100kD)
208	GTGCTGATTC	1640	collagen, type VII, alpha 1 (epidermolysis bullosa, dystrophic, dominant and recessive)
209	CGACCCCACG	169401	apolipoprotein E
210	TTTTGTAACT	256549	nucleotide binding protein 2 (MinD homolog, E. coli)
211	TCTAAGTACG		
212	CTTCCTTGCC	2785	keratin 17
213	CTTCCTTGCC	272572	hemoglobin, alpha 1
214	TTAAGAAGTT	275360	ESTs
215	GCTCTGCTTG	112408	S100 calcium binding protein A7 (psoriasin 1)
216	ATTAAGAGGG		
217	GAGCAGCGCC	112408	S100 calcium binding protein A7 (psoriasin 1)
218	CCTGGGAAGT	12035	ESTs, Weakly similar to 2004399A chromosomal protein [Homo sapiens] [H.sapiens]
219	CCTGGGAAGT	89603	mucin 1, transmembrane
220	CAAACTAACC	75813	polycystic kidney disease 1 (autosomal dominant)
221	CAAACTAACC	153261	immunoglobulin heavy constant mu
222	AAACCCCAAT	8997	Sad1 unc-84 domain protein 1
223	AAACCCCAAT	77735	hypothetical protein FLJ11618
224	GAAATAAAGC	300697	immunoglobulin heavy constant gamma 3 (G3m marker)
225	GAAATAAAGC	111334	ferritin, light polypeptide
226	AAGGGAGCAC	181125	immunoglobulin lambda locus
227	AAGGGAGCAC	8997	Sad1 unc-84 domain protein 1
228	GGAGTGTGCT	9615	myosin, light polypeptide 9, regulatory
229	CATATCATTA	119206	insulin-like growth factor binding protein 7
230	TTTTTAATGT	181307	H3 histone, family 3A
231	TTTTTAATGT	356202	ESTs, Highly similar to S06250 histone H3 [similarity]
232	CTCCCCCAAG		

Table 3. Genes employed for the clustering analysis shown in Fig. 3B

SEQ ID NO:	Tag	Unigene	Gene name
233	CTCCCCCAAA	306886	Homo sapiens cDNA: FLJ23175 fis, clone LNG10438
234	GTTCACATTA	51615	ESTs, Weakly similar to hypothetical protein FLJ20378 [Homo sapiens] [H.sapiens]
235	GTTCACATTA	84298	CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)
236	GTACGTATTC	76325	immunoglobulin J polypeptide, linker protein for immunoglobulin alpha and mu polypeptides
237	GTACGTATTC	146657	ESTs
238	TAAAATATTG	4193	ortholog of mouse integral membrane glycoprotein LIG-1
239	TAATAAAGGT	151604	ribosomal protein S8
240	TAATAAAGGT	374502	ESTs, Highly similar to S25022 ribosomal protein S8, cytosolic
241	CAATAAATGT	163109	ESTs
242	CAATAAATGT	337445	ribosomal protein L37
243	CTCTCACCCT	75108	ribonuclease/angiogenin inhibitor
244	CTCTCACCCT	268189	hypothetical protein FLJ20436
245	GTGCCTAGGG	198166	activating transcription factor 2
246	CCTATTTACT	347969	cytochrome c oxidase subunit IV isoform 1
247	CTGTTGATTG	249495	heterogeneous nuclear ribonucleoprotein A1
248	CTGTTGATTG	356723	ESTs, Highly similar to S04617 heterogeneous ribonuclear particle protein A1
249	GTTGTCTTTG	258798	hypothetical protein FLJ20003
250	GTTGTCTTTG	284394	complement component 3
251	GCTCACCTGT	29647	uncharacterized hematopoietic stem/progenitor cells protein MDS028
252	GCTCACCTGT	159142	lunatic fringe homolog (Drosophila)
253	GTGTAATAAG	232400	heterogeneous nuclear ribonucleoprotein A2/B1
254	CAATGCTGCC	234518	ribosomal protein L23
255	GTGATGGTGT	197345	thyroid autoantigen 70kD (Ku antigen)
256	GTGATGGTGT	3352	histone deacetylase 2
257	TGAGGGAATA	83848	triosephosphate isomerase 1
258	GGCACAGTAA	11270	hypothetical protein MGC2491
259	GGCACAGTAA	49169	KIAA1634 protein
260	GGCTGTACCC	108080	cysteine and glycine-rich protein 1
261	GGCTGTACCC	96908	p53-induced protein
262	AACACAGCCT	170250	complement component 4A
263	AACACAGCCT	278625	complement component 4B
264	CAGTTCTCTG	279921	hypothetical protein MGC8721
265	AAGGACCTAG		
266	TAATAAATGC		
267	CCCTATCACA	150826	RAB25, member RAS oncogene family
268	CGGTTTAATT		
269	TTTCTAGTTT	111894	lysosomal-associated protein transmembrane 4 alpha
270	CTGGAGGCTG	98967	ATPase, H+ transporting, lysosomal V0 subunit a isoform 4
271	CTGGAGGCTG	149152	rhophilin 1
272	CCTAGCTGGA	356332	ESTs, Moderately similar to S71220 peptidylprolyl isomerase (EC 5.2.1.8) ROC2
273	CCTAGCTGGA	342389	peptidylprolyl isomerase A (cyclophilin A)
274	TTACCTCCTT	355815	Homo sapiens, clone MGC:8772 IMAGE:3862861, mRNA, complete cds
275	CAATTAAAAG	36475	Homo sapiens cDNA FLJ36837 fis, clone ASTRO2011422
276	CAATTAAAAG	149923	X-box binding protein 1
277	CCTTTCACAC	278589	general transcription factor II, i
278	CCTTTCACAC	356669	Homo sapiens cDNA FLJ25021 fis, clone CBL01740
279	TTCGGTTGGT	24809	hypothetical protein FLJ10826
280	GGTAGTTTTA	82302	Homo sapiens cDNA FLJ32144 fis, clone PLACE5000105, highly similar to Mus musculus mRNA for heparan sulfate 6-sulfotransferase 2
281	GTAGACACCT	153	ribosomal protein L7
282	TTTAATTTGT	182793	golgi phosphoprotein 2
283	TTTAATTTGT	220689	Ras-GTPase-activating protein SH3-domain-binding protein
284	AAGTTGCTAT	78575	prosaposin (variant Gaucher disease and variant metachromatic leukodystrophy)
285	AAGTTGCTAT	103382	phospholipid scramblase 3
286	GGAATGTACG	429	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subunit 9) isoform 3

Table 3. Genes employed for the clustering analysis shown in Fig. 3B

SEQ ID NO:	Tag	Unigene	Gene name
287	CAAGCAGGAC	179516	integral type I protein
288	TAGGACAACT	367720	ESTs, Highly similar to HSHU33 histone H3.3
289	CACCACGGTG	241471	RNB6
290	TACAGTATGT	170171	glutamate-ammonia ligase (glutamine synthase)
291	CTGTTGGTGA	3463	ribosomal protein S23
292	CTGTTGGTGA	356628	ESTs, Moderately similar to T48317 hypothetical protein F9G14.270
293	TGTATGAATT	25328	Homo sapiens, clone IMAGE:4617948, mRNA
294	TGTATGAATT	28777	H2A histone family, member L
295	CTCGCGCTGG	40369	Homo sapiens cDNA FLJ33345 fis, clone BRACE2003713
296	CTCGCGCTGG	25640	claudin 3
297	GGTGAGACAC	164280	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 6
298	GGTGAGACAC	350927	Homo sapiens cDNA FLJ30227 fis, clone BRACE2001865
299	GGGGTAAGAA	80423	prostatic binding protein
300	GCAGCCATCC	4437	ribosomal protein L28
301	TGCTGGTGTG	298573	KIAA1720 protein
302	TGCTGGTGTG	84883	KIAA0864 protein
303	AGGGCTTCCA	356767	ESTs, Weakly similar to 60S ribosomal protein L10, putative [Arabidopsis thaliana] [A.thaliana]
304	AGGGCTTCCA	29797	ribosomal protein L10
305	GTAGGGGTAA		
306	CTTGAGCAAT	848	FK506 binding protein 4 (59kD)
307	GTCTGGGGCT	75725	thiopurine S-methyltransferase
308	GCCCCCAATA	227751	lectin, galactoside-binding, soluble, 1 (galectin 1)
309	TGGCTGGGAA	172684	vesicle-associated membrane protein 8 (endobrevin)
310	GGGCCCAGGA	25197	STIP1 homology and U-Box containing protein 1
311	GGGCCCAGGA	118983	hypothetical protein FLJ12150
312	CAAGGGCCAA	170160	RAB2, member RAS oncogene family-like
313	GCAAAAGAAA	1265	branched chain keto acid dehydrogenase E1, beta polypeptide (maple syrup urine disease)
314	GCAAAAGAAA	155543	proteasome (prosome, macropain) 26S subunit, non-ATPase, 7 (Mov34 homolog)
315	CTCCACCCGA	82961	Trefoil factor 3
316	AATATGTGGG	98664	ESTs, Moderately similar to COXH HUMAN Cytochrome c oxidase polypeptide VIC precursor [H.sapiens]
317	AATATGTGGG	351875	cytochrome c oxidase subunit VIc
318	GTAGTTACTG	269021	ESTs
319	TGGCAACCTT	279952	glutathione S-transferase subunit 13 homolog
320	TGGCAACCTT	75117	interleukin enhancer binding factor 2, 45kD
321	TGTCATAGTT		
322	GTCCCTGCCT	279837	glutathione S-transferase M2 (muscle)
323	GTCCCTGCCT	301961	glutathione S-transferase M1
324	ATTGTTTATG	181163	high-mobility group (nonhistone chromosomal) protein 17
325	ATTGTTTATG	33317	KIAA1393 protein
326	GCCTGCTGGG	2706	glutathione peroxidase 4 (phospholipid hydroperoxidase)
327	TGCTGCCTGT	118110	bone marrow stromal cell antigen 2
328	TGCTGCCTGT	145477	HCGIV-6 protein
329	GTGACCTCCT	180139	SMT3 suppressor of mif two 3 homolog 2 (yeast)
330	CACGCAATGC	244	amino-terminal enhancer of split
331	CACGCAATGC	21907	histone acetyltransferase
332	CAAACCATCC	65114	keratin 18
333	CAAACCATCC	348292	Homo sapiens cDNA: FLJ22448 fis, clone HRC09541
334	ACCGCCTGTG	79625	chromosome 20 open reading frame 149
335	CTCAACATCT	348311	ribosomal protein, large, P0 pseudogene 2
336	CTCAACATCT	350108	ribosomal protein, large, P0
337	TTGTAATCGT		
338	GTGCCATATT	5337	isocitrate dehydrogenase 2 (NADP+), mitochondrial
339	GTGCCATATT	254709	EST
340	CATTTGTAAT	13999	KIAA0700 protein
341	AGTGCCGTGT	154654	cytochrome P450, subfamily I (dioxin-inducible), polypeptide 1 (glaucoma 3, primary infantile)

Table 3. Genes employed for the clustering analysis shown in Fig. 3B

SEQ ID NO:	Tag	Unigene	Gene name
342	AGTGCCGTGT	76391	myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)
343	ATGGCTGGTA	182426	ribosomal protein S2
344	ATGGCTGGTA	334668	hypothetical protein FLJ23209
345	GGCTTTACCC	119140	eukaryotic translation initiation factor 5A
346	TTGGTGAAGG	75968	thymosin, beta 4, X chromosome
347	TTGGTGAAGG	356629	Homo sapiens cDNA FLJ31414 fis, clone NT2NE2000260, weakly similar to THYMOSIN BETA-4
348	TAGCTCTATG	76549	ATPase, Na+/K+ transporting, alpha 1 polypeptide
349	AATAAAGAGA	28149	hypothetical protein BC010626
350	AATAAAGAGA	337535	ESTs
351	CAAATAAAAA	1116	lymphotoxin beta receptor (TNFR superfamily, member 3)
352	CAAATAAAAA	21198	translocase of outer mitochondrial membrane 70 homolog A (yeast)
353	TACCATCAAT	79877	myotubularin related protein 6
354	TACCATCAAT	169476	glyceraldehyde-3-phosphate dehydrogenase
355	TAAGTAGCAA	111911	ESTs, Weakly similar to T06291 extensin homolog T9E8.80
356	TAAGTAGCAA	239625	integral membrane protein 2B
357	GAAGCAGGAC	180370	cofilin 1 (non-muscle)
358	TTAGCAATAA	74346	hypothetical protein MGC14353
359	TTAGCAATAA	75798	chromosome 20 open reading frame 111
360	CAATGTGTTA	74823	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 1 (7.5kD, MWFE)
361	CAATGTGTTA	181788	ESTs
362	GAGGACCCAA	77313	cyclin-dependent kinase (CDC2-like) 10
363	CCGTGCTCAT	9857	dicarbonyl/L-xylulose reductase
364	GGGTGCTTGG	6551	ATPase, H+ transporting, lysosomal interacting protein 1
365	GTGCAGGGAG	79414	prostate epithelium-specific Ets transcription factor
366	GTGCAGGGAG	180403	STRIN protein
367	TTACTAAATG	155560	calnexin
368	TTACTAAATG	7917	DKFZP564K247 protein
369	GAAATACAGT	67201	5',3'-nucleotidase, cytosolic
370	GAAATACAGT	343475	cathepsin D (lysosomal aspartyl protease)
371	CAAATAAAAT	71465	squalene epoxidase
372	TGCATCTGGT	75410	heat shock 70kD protein 5 (glucose-regulated protein, 78kD)
373	TTTCAGGGGA		
374	TTTGGTGTTT	83190	fatty acid synthase
375	TACCTCTGAT	2962	S100 calcium binding protein P
376	TACCTCTGAT	263455	ESTs, Weakly similar to hypothetical protein FLJ20489 [Homo sapiens] [H.sapiens]
377	GGCCAGCCCT	155455	phosphofructokinase, liver
378	GGCCAGCCCT	79	hypothetical protein MGC15429
379	GCTTTGATGA	89649	epoxide hydrolase 1, microsomal (xenobiotic)
380	GCTTTGATGA	279681	heterogeneous nuclear ribonucleoprotein H3 (2H9)
381	AATAAAGGCT	1815	myosin, light polypeptide 3, alkali; ventricular, skeletal, slow
382	AATAAAGGCT	179735	ras homolog gene family, member C
383	CCTTTGCCCT		
384	CACTTCAAGG	77667	lymphocyte antigen 6 complex, locus E
385	TTCATACACC		
386	TCTGTACACC	182740	ribosomal protein S11
387	CCATTGCACT	194382	ataxia telangiectasia mutated (includes complementation groups A, C and D)
388	CCATTGCACT	244378	solute carrier family 2 (facilitated glucose transporter), member 6
389	AAATAAAGAA	14841	ESTs
390	AAATAAAGAA	355733	microsomal glutathione S-transferase 1
391	GGGTTGGCTT	73818	ubiquinol-cytochrome c reductase hinge protein
392	ACTTTTTCAA	133430	ESTs
393	ACTTTTTCAA	246500	EST
394	CCCATCGTCC		
395	GCGGCTTTCC	278431	SCO cytochrome oxidase deficient homolog 2 (yeast)
396	GGGAAGCAGA		

Table 3. Genes employed for the clustering analysis shown in Fig. 3B

SEQ ID NO:	Tag	Unigene	Gene name
397	CTGACCTGTG	77961	major histocompatibility complex, class I, B
398	CTGACCTGTG	181244	major histocompatibility complex, class I, A
399	GTAAGTGTAC		
400	TAGTTGGAAA	1119	nuclear receptor subfamily 4, group A, member 1
401	ATTTTCTAAA	91011	anterior gradient 2 homolog (Xenepus laevis)
402	TGCTAAAAAA	146550	myosin, heavy polypeptide 9, non-muscle
403	TGCTAAAAAA	313761	ESTs
404	GGAATAAATT		
405	GTGTGTAAAA	291904	accessory protein BAP31
406	AGAAAAAAAA	153834	pumilio homolog 1 (Drosophila)
407	AGAAAAAAAA	254105	enolase 1, (alpha)
408	TCAAAAAAAA	10846	polyamine N-acetyltransferase
409	TCAAAAAAAA	333524	hypothetical protein MGC13064
410	CTAAAAAAAA	9873	likely homolog of rat kinase D-interacting substance of 220 kDa
411	CTAAAAAAAA	54457	CD81 antigen (target of antiproliferative antibody 1)
412	CAAAAAAAAA	126906	hypothetical protein FLJ12598
413	CAAAAAAAAA	234355	hypothetical protein FLJ22569
414	GACTCACTTT	699	peptidylprolyl isomerase B (cyclophilin B)
415	AGTTTCCCAA	312644	sulfotransferase family, cytosolic, 1C, member 2
416	AGTTTCCCAA	279929	gp25L2 protein
417	GCAAAAAAAA	4746	hypothetical protein FLJ21324
418	GCAAAAAAAA	91579	similar to HYPOTHETICAL 34.0 KDA PROTEIN ZK795.3 IN CHROMOSOME IV
419	CACTTGCCCT	14779	acetyl-Coenzyme A synthetase 2 (ADP forming)
420	CACTTGCCCT	15977	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 9 (22kD, B22)
421	CTTAATCCTG	298275	solute carrier family 38, member 2
422	AAAAAAAAAA	78713	solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 3
423	AAAAAAAAAA	10235	chromosome 5 open reading frame 4
424	GAAAAAAAAA	12185	protein phosphatase 1, regulatory (inhibitor) subunit 16A
425	GAAAAAAAAA	99843	DKFZP586N0721 protein
426	GGGGACTGAA	438	mesenchyme homeo box 1
427	GGGGACTGAA	3709	low molecular mass ubiquinone-binding protein (9.5kD)
428	TTGAATTCCC	171921	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C
429	GCTTTTTAGA	251064	high-mobility group (nonhistone chromosomal) protein 14
430	GCTTTTTAGA	356285	ESTs, Highly similar to HG14 HUMAN Nonhistone chromosomal protein HMG-14 [H.sapiens]
431	TTTCTGTTAA	12101	hypothetical protein LOC51242
432	TGATCTCCAA	11050	F-box only protein 9
433	TGATCTCCAA	83190	fatty acid synthase
434	AAAGTCTAGA	82932	cyclin D1 (PRAD1: parathyroid adenomatosis 1)
435	CCCTACCCTG	75736	apolipoprotein D
436	TACATAATTA	240443	multiple endocrine neoplasia I
437	TTCAATAAAA	2012	transcobalamin I (vitamin B12 binding protein, R binder family)
438	TTCAATAAAA	177592	ribosomal protein, large, P1
439	TAAGGAGCTG	299465	ribosomal protein S26
440	TAAGGAGCTG	355957	ESTs, Highly similar to RS26 HUMAN 40S ribosomal protein S26 [H.sapiens]
441	TAAAAAAAAA	80612	ubiquitin-conjugating enzyme E2A (RAD6 homolog)
442	TAAAAAAAAA	244621	ribosomal protein S14
443	TCTGTTTATC	180394	signal recognition particle 14kD (homologous Alu RNA binding protein)
444	TCTGTTTATC	355573	ESTs, Highly similar to S34196 signal recognition particle 14K chain
445	GTAAAAAAAA	77495	UBX domain-containing 2
446	GTAAAAAAAA	279887	aryl hydrocarbon receptor interacting protein-like 1
447	CCCCAGTTGC	120811	ESTs
448	CCCCAGTTGC	74451	calpain, small subunit 1
449	TGTACCTGTA	249922	EST
450	TGTACCTGTA	334842	tubulin, alpha, ubiquitous
451	GAACACATCC	252723	ribosomal protein L19
452	AATAGTTGTG		

Table 3. Genes employed for the clustering analysis shown in Fig. 3B

SEQ ID NO:	Tag	Unigene	Gene name
453	AACTAAAAAA	3297	ribosomal protein S27a
454	AACTAAAAAA	55921	glutamyl-prolyl-tRNA synthetase
455	TAGGTTGTCT	279860	tumor protein, translationally-controlled 1
456	TAGGTTGTCT	374596	ESTs, Highly similar to S06590 IgE-dependent histamine-releasing factor
457	TTAAAAAAAA	19054	hypothetical protein PRO2521
458	TTAAAAAAAA	78825	matrin 3
459	AACTAACAAA	25996	ESTs, Moderately similar to UQHUR7 ubiquitin
460	AACTAACAAA	3297	ribosomal protein S27a
461	CAAGGGCTTG	156764	RAP1B, member of RAS oncogene family
462	AAGGCAATTT	301626	Homo sapiens cDNA FLJ11739 fis, clone HEMBA1005497
463	AAGGCAATTT	164170	vascular Rab-GAP/TBC-containing
464	CTCCTCACCT	93213	BCL2-antagonist/killer 1
465	CTCCTCACCT	119122	ribosomal protein L13a
466	GACTCTGGTG	334859	histone methyltransferase DOT1L
467	GACTCTGGTG	356189	Homo sapiens, ribosomal protein S15a, clone MGC:44895 IMAGE:5580542, mRNA, complete cds
468	ATTCTCCAGT	234518	ribosomal protein L23
469	AAAAAACCCA	111680	endosulfine alpha
470	TGATAATTCA	171625	hypothetical protein MGC14697
471	GGGCTGGGGT	90436	sperm associated antigen 7
472	GGGCTGGGGT	350068	ribosomal protein L29
473	GCTTAACCTG	77508	glutamate dehydrogenase 1
474	GGATTTGGCC	82506	KIAA1254 protein
475	GGATTTGGCC	343426	ESTs
476	TGCACGTTTT	169793	ribosomal protein L32
477	GCATAATAGG	356482	ESTs, Weakly similar to putative 60S ribosomal protein L21 [Arabidopsis thaliana] [A.thaliana]
478	GCATAATAGG	350077	ribosomal protein L21
479	GCACAAGAAG	289721	growth arrest-specific 5
480	TAAACTGTTT	244621	ribosomal protein S14
481	TCAGATCTTT	108124	ribosomal protein S4, X-linked
482	GACAAAAAAA	343665	ribosomal protein S15a
483	GACAAAAAAA	356505	ESTs, Moderately similar to RS1A ARATH 40S ribosomal protein S15A [A.thaliana]
484	GGAACAAACA	197345	thyroid autoantigen 70kD (Ku antigen)
485	GGAACAAACA	286124	CD24 antigen (small cell lung carcinoma cluster 4 antigen)
486	CTAACTTCGT	14838	likely ortholog of mouse NPC derived proline rich protein 1
487	GCTCAGCTGG	223241	eukaryotic translation elongation factor 1 delta (guanine nucleotide exchange protein)
488	TGGCGTGGCC	8854	Pvt1 oncogene homolog, MYC activator (mouse)
489	AGCCAAAAAA	235768	NK inhibitory receptor precursor
490	AGCCAAAAAA	89388	Homo sapiens cDNA FLJ31372 fis, clone NB9N42000281
491	TGGCGTACGG		
492	GGAGCGTGGG	286226	myosin IC
493	ACAGCGGCAA	323462	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 30
494	ACAGCGGCAA	349499	desmoplakin (DPI, DPII)
495	TCAAGTTCAC	351928	Homo sapiens mRNA full length insert cDNA clone EUROIMAGE 1977059
496	GGAAGCACGG	355544	ESTs, Weakly similar to T05691 multiubiquitin chain-binding protein MBP1
497	GGAAGCACGG	148495	proteasome (prosome, macropain) 26S subunit, non-ATPase, 4
498	CAGTTACAAA	7910	RING1 and YY1 binding protein
499	CAGTTACAAA	312857	ESTs
500	CAGGACAGTT	78305	RAB2, member RAS oncogene family
501	GGGGAAATCG	76293	thymosin, beta 10
502	CAAATCCAAA	227400	mitogen-activated protein kinase kinase kinase kinase 3
503	TCAGAAGTTT	243901	Homo sapiens mRNA; cDNA DKFZp564C1563 (from clone DKFZp564C1563)
504	AAAGTTCTCA	284243	transmembrane 4 superfamily member tetraspan NET-6
505	AAGGATGCCA	169946	GATA binding protein 3
506	AAGGATGCCA	104823	EST
507	GAGGGCCGGT	36727	H2A histone family, member J
508	CAGCAGAAGC	323806	small EDRK-rich factor 2

Table 3. Genes employed for the clustering analysis shown in Fig. 3B

SEQ ID NO:	Tag	Unigene	Gene name
509	CAGCAGAAGC	343261	histocompatibility (minor) 13
510	CCTCCAGCTA	242463	keratin 8
511	CCTCCAGCTA	356123	ESTs, Moderately similar to I37982 Keratin 8
512	GCCTTCCAAT	76053	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 5 (RNA helicase, 68kD)
513	GGGAGCCCGG	183986	poliovirus receptor-related 2 (herpesvirus entry mediator B)
514	GCTCCCAGAC	5097	synaptogyrin 2
515	GCAGGGCCTC	301350	FXYD domain-containing ion transport regulator 3
516	TTGGAGATCT	50098	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 4 (9kD, MLRQ)
517	GGAAAAAAAA	177530	ATP synthase, H+ transporting, mitochondrial F1 complex, epsilon subunit
518	GGAAAAAAAA	198271	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 10 (42kD)
519	AAGAAAACTG	330208	crystallin, zeta (quinone reductase)-like 1
520	AAGAAAACTG	322735	KIAA1522 protein
521	GACATCAAGT	182265	keratin 19
522	GCAGTGGCCT	184276	solute carrier family 9 (sodium/hydrogen exchanger), isoform 3 regulatory factor 1
523	GCAGTGGCCT	161166	KIAA1094 protein
524	CGCCGACGAT	265827	interferon, alpha-inducible protein (clone IFI-6-16)
525	ATGTCTTTTC	1516	insulin-like growth factor binding protein 4
526	ATGTCTTTTC	59483	leucine-rich repeat-containing G protein-coupled receptor 6
527	GCCGTCGGAG	265827	interferon, alpha-inducible protein (clone IFI-6-16)
528	CGGACTCACT	84700	serologically defined colon cancer antigen 28
529	ACGCAGGGAG	279789	glucose phosphate isomerase
530	CCAGGGGAGA	254105	enolase 1, (alpha)
531	CCAGGGGAGA	278613	interferon, alpha-inducible protein 27
532	AAGAAAACCT	100686	anterior gradient protein 3
533	AAGAAAACCT	274319	hypothetical protein FLJ10509
534	AGATTCAAAC	14368	SH3 domain binding glutamic acid-rich protein like
535	TGGGGAGAGG		
536	CCAAACGTGT	181307	H3 histone, family 3A
537	CCAAACGTGT	367720	ESTs, Highly similar to HSHU33 histone H3.3
538	AAGCCTAAAA	79136	LIV-1 protein, estrogen regulated
539	GTGCTGAATG	77385	myosin, light polypeptide 6, alkali, smooth muscle and non-muscle
540	GTGCTGAATG	120260	immunoglobulin superfamily receptor translocation associated 1
541	AACGCGGCCA	60300	hypothetical protein MGC17552
542	AACGCGGCCA	73798	macrophage migration inhibitory factor (glycosylation-inhibiting factor)
543	GGCAACGTGG	300954	Huntingtin interacting protein K
544	GGCAACGTGG	31608	transient receptor potential cation channel, subfamily M, member 4
545	CGCCGCGGTG	4835	eukaryotic translation initiation factor 3, subunit 8 (110kD)
546	GTGACCACGG	299882	ESTs, Highly similar to N-methyl-D-aspartate receptor 2C subunit precursor [Homo sapiens] [H.sapiens]
547	CCGACGGGCG		
548	GGTGGCACTC	77273	ras homolog gene family, member A
549	GGTGGCACTC	77550	p53-regulated DDA3
550	GGGATCAAGG	9265	mitochondrial ribosomal protein L24
551	TGGAGTGGAG	3764	guanylate kinase 1
552	TGCCTCTGCG		
553	TCCCTGGCTG	78575	prosaposin (variant Gaucher disease and variant metachromatic leukodystrophy)
554	TCCCTGGCTG	166160	acetyl-Coenzyme A acyltransferase 1 (peroxisomal 3-oxoacyl-Coenzyme A thiolase)
555	GACGACACGA	153177	ribosomal protein S28
556	GACGACACGA	374547	ESTs, Moderately similar to RS28 ARATH 40S ribosomal protein S28 [A.thaliana]
557	GTGCTGGACC	20977	ganglioside-induced differentiation-associated protein 1-like 1
558	GTGCTGGACC	179774	proteasome (prosome, macropain) activator subunit 2 (PA28 beta)
559	GCAGGCCAAG	69771	B-factor, properdin
560	GCAGGCCAAG	159505	RAB30, member RAS oncogene family
561	TGCCTGCACC	135084	cystatin C (amyloid angiopathy and cerebral hemorrhage)
562	TCAGCCTTCT	112165	Homo sapiens cDNA FLJ12198 fis, clone MAMMA1000876
563	TCAGCCTTCT	179986	flotillin 1

Table 3. Genes employed for the clustering analysis shown in Fig. 3B

SEQ ID NO:	Tag	Unigene	Gene name
564	TAGAAAAATA	79194	cAMP responsive element binding protein 1
565	TAGAAAAATA	279789	glucose phosphate isomerase
566	AAGACAGTGG	3352	histone deacetylase 2
567	AAGACAGTGG	296290	ribosomal protein L37a
568	TGTGCTAAAT	250895	ribosomal protein L34
569	TGTGCTAAAT	11387	KIAA1453 protein
570	TCTCCATACC		
571	GGCAAGAAGA	83321	neuromedin B
572	GGCAAGAAGA	111611	ribosomal protein L27
573	GAAAAATTTA	169248	cytochrome c
574	TTGGTCCTCT	356796	Homo sapiens E1BP1 pseudogene, mRNA sequence
575	TTGGTCCTCT	356795	ribosomal protein L41
576	GTGTGGGGGG	2340	junction plakoglobin
577	GTGTGGGGGG	117484	ESTs
578	CGTGGGTGGG	202833	heme oxygenase (decycling) 1
579	GCGACGAGGC	2017	ribosomal protein L38
580	GCCGTTCTTA		
581	ACCCGCCGGG		
582	GGCCTGCTGC	280792	hypothetical protein FLJ12387 similar to kinesin light chain
583	GGCCTGCTGC	9634	hypothetical protein BC009925
584	GGTTTGGCTT	73818	ubiquinol-cytochrome c reductase hinge protein
585	TCAGTTTGTC	121397	ESTs
586	TCAGTTTGTC	15318	HS1 binding protein
587	GGTCAGTCGG		
588	CTAACTAGTT		
589	AAGGTGGAGG	76171	CCAAT/enhancer binding protein (C/EBP), alpha
590	AAGGTGGAGG	163593	ribosomal protein L18a
591	AGGCTACGGA	119122	ribosomal protein L13a
592	AGGCTACGGA	356678	ESTs, Weakly similar to T07697 ribosomal protein L13a, cytosolic
593	GAAGTTATGA	4112	t-complex 1
594	TCACAAGCAA	32916	nascent-polypeptide-associated complex alpha polypeptide
595	GCGCTGGAGT	241432	ESTs, Highly similar to c380A1.1b [H.sapiens]
596	GCGCTGGAGT	110695	hypothetical protein MGC3133
597	GGACCACTGA	119598	ribosomal protein L3
598	GGACCACTGA	356258	ESTs, Weakly similar to ribosomal protein [Arabidopsis thaliana] [A.thaliana]
599	GCGGTGAGGT	203910	small glutamine-rich tetratricopeptide repeat (TPR)-containing
600	CAATAAACTG	150580	putative translation initiation factor
601	CAATAAACTG	297112	ESTs
602	AGGAAAGCTG	227591	hypothetical protein FLJ11088
603	AGGAAAGCTG	343443	ribosomal protein L36
604	CTGGGTTAAT	356647	ESTs
605	CTGGGTTAAT	298262	ribosomal protein S19
606	AAGGAGATGG	164170	vascular Rab-GAP/TBC-containing
607	AAGGAGATGG	355990	ESTs, Highly similar to R5HU31 ribosomal protein L31
608	ACATCATCGA	182979	ribosomal protein L12
609	ACATCATCGA	356318	ESTs, Weakly similar to T45883 60S RIBOSOMAL PROTEIN L12-like
610	ATTATTTTTC	153	ribosomal protein L7
611	ATTATTTTTC	356593	ribosomal protein L7
612	TAGTTGAAGT	131255	ubiquinol-cytochrome c reductase binding protein
613	CCAGAACAGA	79006	deoxythymidylate kinase (thymidylate kinase)
614	CCAGAACAGA	334807	ribosomal protein L30
615	GCATTTAAAT	275959	eukaryotic translation elongation factor 1 beta 2
616	GCATTTAAAT	356184	ESTs, Weakly similar to elongation factor 1-beta, putative [Arabidopsis thaliana] [A.thaliana]
617	GAAAAATGGT	181357	laminin receptor 1 (67kD, ribosomal protein SA)
618	GAAAAATGGT	356267	Homo sapiens laminin receptor-like protein LAMRL5 mRNA, complete cds
619	GGTTGGCAGG	3745	milk fat globule-EGF factor 8 protein

Table 3. Genes employed for the clustering analysis shown in Fig. 3B

SEQ ID NO:	Tag	Unigene	Gene name
620	GGTTGGCAGG	17908	origin recognition complex, subunit 1-like (yeast)
621	GTGAAGGCAG	77039	ribosomal protein S3A
622	GTGAAGGCAG	356568	ESTs, Weakly similar to Putative S-phase-specific ribosomal protein [Arabidopsis thaliana] [A.thaliana]
623	TTGCGTTGCG		
624	ATCTCAGCTC	8036	RAB3D, member RAS oncogene family
625	ATCTCAGCTC	29736	TNF receptor-associated factor 5
626	AAAAAATTCA	254271	hypothetical protein MGC24009
627	TGGCCCCACC	146662	Homo sapiens cDNA FLJ36928 fis, clone BRACE2005216, weakly similar to Xenopus laevis bicaudal-C (Bic C) mRNA
628	TGGCCCCACC	198281	pyruvate kinase, muscle
629	TCCATCTGTT	252189	syndecan 4 (amphiglycan, ryudocan)
630	CAACTGGAGT	166011	catenin (cadherin-associated protein), delta 1
631	CAACTGGAGT	352566	cytochrome P450 monooxygenase
632	GCCCAGCTGG	12479	associated molecule with the SH3 domain of STAM
633	GCCCAGCTGG	334798	hypothetical protein FLJ20897
634	GACGGCGCAG	73946	endothelial cell growth factor 1 (platelet-derived)
635	ATGAAACCCC	75470	chromosome 1 open reading frame 29
636	ATGAAACCCC	226396	hypothetical protein FLJ11126
637	AGCCACCGCA	242	glucose-6-phosphatase, catalytic (glycogen storage disease type I, von Gierke disease)
638	AGCCACCGCA	244482	M-phase phosphoprotein, mpp8
639	CCCAGCTAAT	73809	arachidonate 15-lipoxygenase
640	CCCAGCTAAT	200395	centromere protein H
641	GTGAAACCCC	44396	coronin, actin binding protein, 2A
642	GTGAAACCCC	323949	kangai 1 (suppression of tumorigenicity 6, prostate; CD82 antigen (R2 leukocyte antigen, antigen detected by monoclonal and antibody IA4))
643	GTGAAACCCT	289053	CAP-binding protein complex interacting protein 2
644	GTGAAACCCT	52644	src family associated phosphoprotein 2
645	GAGAAACCCC	5719	chromosome condensation-related SMC-associated protein 1
646	GAGAAACCCC	114318	hypothetical protein MGC16385
647	GTGAAACCTT	365695	Homo sapiens cDNA FLJ11083 fis, clone PLACE1005232
648	GTGAAACCTT	264636	FK506 binding protein 14 (22 kDa)
649	GTGAAACTCC	75410	heat shock 70kD protein 5 (glucose-regulated protein, 78kD)
650	GTGAAACTCC	256158	hypothetical protein BC018697
651	GTGAAATCCC	274448	hypothetical protein FLJ11029
652	GTGAAATCCC	287587	Homo sapiens cDNA FLJ13671 fis, clone PLACE1011729
653	AACCCGGGAG	118744	KIAA0408 gene product
654	AACCCGGGAG	173936	interleukin 10 receptor, beta
655	GTGGCGGGCA	6874	KIAA0472 protein
656	GTGGCGGGCA	169813	hypothetical protein FLJ23040
657	TTGCCCAGGC	9711	novel protein
658	TTGCCCAGGC	286124	CD24 antigen (small cell lung carcinoma cluster 4 antigen)
659	GTGGTGGGTG	289020	Homo sapiens cDNA FLJ11553 fis, clone HEMBA1003034
660	GTGGTGGGTG	171731	solute carrier family 14 (urea transporter), member 1 (Kidd blood group)
661	CCTGTAATCC	181874	interferon-induced protein with tetratricopeptide repeats 4
662	CCTGTAATCC	292154	stromal cell protein
663	AGCCACTGTG	147313	similar to CMRF35 antigen precursor (CMRF-35)
664	AGCCACTGTG	348642	Homo sapiens FGF2-associated protein GAFA1 (GAFA1) mRNA, complete cds
665	GTGGCAGGCA	13255	KIAA0930 protein
666	GTGGCAGGCA	47334	reserved
667	GTAAAACCCC	12106	hypothetical protein MGC20496
668	GTAAAACCCC	256278	tumor necrosis factor receptor superfamily, member 1B
669	CCTGGCTAAT	274170	Opa-interacting protein 2
670	CCTGGCTAAT	117062	apoptosis-inducing factor (AIF)-homologous mitochondrion-associated inducer of death
671	GTGAAATCCT	301509	Homo sapiens cDNA FLJ12339 fis, clone MAMMA1002250
672	GTGAAATCCT	9280	proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional protease 2)

Table 3. Genes employed for the clustering analysis shown in Fig. 3B

SEQ ID NO:	Tag	Unigene	Gene name
673	GTGGCACGTG	29759	polymerase I and transcript release factor
674	GTGGCACGTG	306850	Homo sapiens cDNA: FLJ22796 fis, clone KAIA2544
675	GTGGCTCACA	270134	hypothetical protein FLJ20280
676	GTGGCTCACA	124813	hypothetical protein MGC14817
677	TGCCTGTAAT	349344	hypothetical protein BC001573
678	TGCCTGTAAT	342655	Homo sapiens cDNA FLJ13289 fis, clone OVARC1001170
679	CCACTGCACT	14992	hypothetical protein FLJ11151
680	CCACTGCACT	107003	enhancer of invasion 10
681	AGAATTGCTT	78060	phosphorylase kinase, beta
682	AGAATTGCTT	190311	nephrosis 1, congenital, Finnish type (nephrin)
683	ATCTTGGCTC	75859	mitochondrial ribosomal protein L49
684	ATCTTGGCTC	129228	galactokinase 2
685	TTGGCCAGGA	146668	KIAA1253 protein
686	TTGGCCAGGA	233335	KIAA1465 protein
687	TTGACCAGGC	193384	putatative 28 kDa protein
688	TTGACCAGGC	194351	coagulation factor II (thrombin) receptor-like 2
689	ATCCGCCCGC	352382	PI-3-kinase-related kinase SMG-1
690	ATCCGCCCGC	355762	Homo sapiens cDNA FLJ35653 fis, clone SPLEN2013690
691	AGCCACCACG	57735	scavenger receptor expressed by endothelial cells
692	AGCCACCACG	2593	phosphodiesterase 6B, cGMP-specific, rod, beta (congenital stationary night blindness 3, autosomal dominant)
693	GTGAAACCCG	278577	Homo sapiens mRNA; cDNA DKFZp564P073 (from clone DKFZp564P073)
694	GTGAAACCCG	302075	Homo sapiens cDNA FLJ12365 fis, clone MAMMA1002392
695	CCCGGCTAAT	273759	Homo sapiens cDNA FLJ11905 fis, clone HEMBB1000050
696	CCCGGCTAAT	325116	JM11 protein
697	GTGAAACCCA	17311	hypothetical protein FLJ20004
698	GTGAAACCCA	241205	peroxisomal membrane protein 4 (24kD)
699	GTAAAACCCT	281680	peroxisomal trans 2-enoyl CoA reductase; putative short chain alcohol dehydrogenase
700	GTAAAACCCT	282797	Homo sapiens cDNA FLJ31194 fis, clone KIDNE2000510
701	GTGAAACTCT	188853	Homo sapiens cDNA FLJ12246 fis, clone MAMMA1001343
702	GTGAAACTCT	333449	Homo sapiens cDNA FLJ12170 fis, clone MAMMA1000664
703	GTGGCGGGTG	257584	Homo sapiens cDNA FLJ12138 fis, clone MAMMA1000331
704	GTGGCGGGTG	296697	Homo sapiens cDNA FLJ12093 fis, clone HEMBB1002603
705	GTGGCAGGTG	280380	aminopeptidase
706	GTGGCAGGTG	333480	Homo sapiens cDNA FLJ13757 fis, clone PLACE3000405
707	GCAAAACCCT	10844	leucine-rich alpha-2-glycoprotein
708	GCAAAACCCT	121576	myosin IB
709	GCAAAACCCC	86412	chromosome 9 open reading frame 5
710	GCAAAACCCC	129708	tumor necrosis factor (ligand) superfamily, member 14
711	AGGTCAGGAG	209065	hypothetical protein FLJ14225
712	AGGTCAGGAG	212414	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3E
713	AGCCACCGTG	156051	KIAA1443 protein
714	AGCCACCGTG	240845	DKFZP434D146 protein
715	GTGGCACACA	129057	breast carcinoma amplified sequence 1
716	GTGGCACACA	207251	nucleolar autoantigen (55kD) similar to rat synaptonemal complex protein
717	ATCTCGGCTC	156942	hypothetical protein BC017947
718	ATCTCGGCTC	271285	KIAA1510 protein
719	TTGGCCAGAC	91728	polymyositis/scleroderma autoantigen 1 (75kD)
720	TTGGCCAGAC	374296	hypothetical protein similar to KIAA0187 gene product
721	GTGGCAGGCG	48604	DKFZP434B168 protein
722	GTGGCAGGCG	53985	glycoprotein 2 (zymogen granule membrane)
723	CACCTGTAAT	175613	claspin
724	CACCTGTAAT	287473	hypothetical protein FLJ11996
725	TTGGCCAGGG	321687	F-box protein FBX30
726	TTGGCCAGGG	322840	Homo sapiens, Similar to protein tyrosine phosphatase-like (proline instead of catalytic arginine), member a,

Table 3. Genes employed for the clustering analysis shown in Fig. 3B

SEQ ID NO:	Tag	Unigene	Gene name
727	GAGAAACCCT	321149	hypothetical protein FLJ10257
728	GAGAAACCCT	274279	hypothetical protein FLJ10314
729	GCGAAACCCT	103189	lipopolysaccharide specific response-68 protein
730	GCGAAACCCT	225084	hypothetical protein FLJ14280
731	GTGAAACCTC	168159	bifunctional apoptosis regulator
732	GTGAAACCTC	334526	hypothetical protein MGC14126
733	GCGAAACCCC	30211	hypothetical protein FLJ22313
734	GCGAAACCCC	288945	hypothetical protein FLJ13448
735	AGCCACCGCG	122660	RAB, member of RAS oncogene family-like 2A
736	AGCCACCGCG	355874	RAB, member of RAS oncogene family-like 2B
737	CGCCTGTAAT	154443	MCM4 minichromosome maintenance deficient 4 (S. cerevisiae)
738	CGCCTGTAAT	287594	hypothetical protein FLJ13769
739	GTGGCGGGCG	22926	KIAA0795 protein
740	GTGGCGGGCG	181780	hypothetical protein FLJ20241
741	AACCTGGGAG	105658	DNA fragmentation factor, 45 kD, alpha polypeptide
742	AACCTGGGAG	334638	hypothetical protein MGC16175
743	GCTTTCTCAC		
744	CTTGTAATCC	183253	nucleolar RNA-associated protein
745	CTTGTAATCC	231119	protocadherin beta 9
746	TCTGTAATCC	272216	glycoprotein VI (platelet)
747	TCTGTAATCC	142	sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1
748	CCTATAATCC	86228	TRIAD3 protein
749	CCTATAATCC	189658	CGI-149 protein
750	TAATCCCAGC	12496	Homo sapiens cDNA FLJ23834 fis, clone KAIA2087
751	TAATCCCAGC	278941	PRO0628 protein
752	TGCCTGTAGT	48469	LIM domains containing 1
753	TGCCTGTAGT	274201	chromosome 1 open reading frame 33
754	AGGGTGTTTT	75842	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A
755	AGGGTGTTTT	160416	ESTs
756	CCAGGGCAAC	240443	multiple endocrine neoplasia I
757	ATTGTGCCAC	22151	neurolysin (metallopeptidase M3 family)
758	ATTGTGCCAC	38761	Homo sapiens cDNA: FLJ21564 fis, clone COL06452
759	CCTGTAATCT	199067	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)
760	CCTGTAATCT	3530	FUS interacting protein (serine-arginine rich) 1
761	GTGGTGGGCA	99975	cholinergic receptor, nicotinic, delta polypeptide
762	GTGGTGGGCA	374536	isovaleryl Coenzyme A dehydrogenase
763	TACCCTAAAA	165662	KIAA0675 gene product
764	TACCCTAAAA	268971	Homo sapiens clone IMAGE:212461, mRNA sequence
765	ATGGTGGGGG	343586	zinc finger protein 36, C3H type, homolog (mouse)
766	ACCCTTGGCC		
767	GTGAAAACCC	127305	agmatine ureohydrolase (agmatinase)
768	GTGAAAACCC	351029	Homo sapiens cDNA FLJ31803 fis, clone NT2RI2009101
769	ATCCACCCGC	145381	general transcription factor IIE, polypeptide 1 (alpha subunit, 56kD)
770	ATCCACCCGC	53263	nucleoporin Nup43
771	TTAGCCAGGA	196270	folate transporter/carrier
772	TTAGCCAGGA	350692	Homo sapiens cDNA FLJ32756 fis, clone TESTI2001758
773	ATGAAACCCT	31330	Homo sapiens clone HQ0319
774	ATGAAACCCT	187991	SOCS box-containing WD protein SWiP-1
775	GTGGCTCACG	3454	KIAA1821 protein
776	GTGGCTCACG	127649	zinc finger protein 297B
777	TTGGCCAGGC	118194	debranching enzyme homolog 1 (S. cerevisiae)
778	TTGGCCAGGC	274382	protein kinase, interferon-inducible double stranded RNA dependent
779	TTGGTCAGGC	154069	melan-A
780	TTGGTCAGGC	172012	hypothetical protein DKFZp434J037
781	TTGTCCAGGC	99423	ATP-dependent RNA helicase
782	TTGTCCAGGC	51305	v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)

Table 3. Genes employed for the clustering analysis shown in Fig. 3B

SEQ ID NO:	Tag	Unigene	Gene name
783	CTTAATCTTG	75462	BTG family, member 2
784	CTTAATCTTG	237356	stromal cell-derived factor 1
785	TGGGGTTCTT	62954	ferritin, heavy polypeptide 1
786	TGGGGTTCTT	272499	dehydrogenase/reductase (SDR family) member 2
787	AAGAAGATAG	350046	ribosomal protein L23a
788	AAGAAGATAG	356007	ESTs, Highly similar to RL2B HUMAN 60S ribosomal protein L23a [H.sapiens]
789	AGAATCGCTT	16165	expressed in activated T/LAK lymphocytes
790	AGAATCGCTT	75887	coatomer protein complex, subunit alpha
791	CCTGTAGTCC	51305	v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)
792	CCTGTAGTCC	77510	hypothetical protein FLJ10520
793	AGCCACCACA	5999	hypothetical protein FLJ10298
794	AGCCACCACA	8768	hypothetical protein FLJ10849
795	ATTGCACCAC	210778	hypothetical protein FLJ10989
796	ATTGCACCAC	287948	Homo sapiens cDNA FLJ11405 fis, clone HEMBA1000769
797	CCACTGTACT	287515	hypothetical protein FLJ12331
798	CCACTGTACT	288537	Homo sapiens cDNA FLJ12199 fis, clone MAMMA1000880
799	CTGTACTTGT	75678	FBJ murine osteosarcoma viral oncogene homolog B
800	CCATTCTCCT	98711	hypothetical protein BC006136
801	CCATTCTCCT	271752	3'(2'), 5'-bisphosphate nucleotidase 1
802	GTGGTGGGCG	73614	solute carrier family 31 (copper transporters), member 1
803	GTGGTGGGCG	287522	Homo sapiens cDNA FLJ12364 fis, clone MAMMA1002384
804	AGCCACTGCG	193914	KIAA0575 gene product
805	AGCCACTGCG	356075	ninjurin 2
806	GCCGGCTCAT		
807	GCTCACTGCA	93523	peptidylprolyl isomerase (cyclophilin)-like 2
808	GCTCACTGCA	117572	chemokine binding protein 2
809	CCTGTGGTCC	120769	Homo sapiens cDNA FLJ20463 fis, clone KAT06143
810	CCTGTGGTCC	243804	Homo sapiens cDNA FLJ13800 fis, clone THYRO1000156
811	GGAGGCTGAG	306189	DKFZP434F1735 protein
812	GGAGGCTGAG	185973	degenerative spermatocyte homolog, lipid desaturase (Drosophila)
813	AGAATCACTT	130815	hypothetical protein FLJ21870
814	AGAATCACTT	192127	Homo sapiens, clone MGC:32020 IMAGE:4620233, mRNA, complete cds
815	CCTGTAATTC	129908	kinesin family member 1B
816	CCTGTAATTC	306678	hypothetical protein FLJ14326
817	AGCCACTGCA	4295	proteasome (prosome, macropain) 26S subunit, non-ATPase, 12
818	AGCCACTGCA	173508	P3ECSL
819	AACCCAGGAG	262150	hypothetical protein FLJ22814
820	AACCCAGGAG	75813	polycystic kidney disease 1 (autosomal dominant)
821	AAGCCAGGAC	10326	coatomer protein complex, subunit epsilon
822	GACCTCCTGC	119324	kinesin-like 4
823	GACCTCCTGC	89449	mitogen-activated protein kinase kinase kinase 11
824	CTGCCAAGTT	75873	zyxin
825	GTTCGTGCCA	195464	filamin A, alpha (actin binding protein 280)
826	GCGCAGAGGT	356795	ribosomal protein L41
827	GCCGTGTCCG	356666	ESTs, Highly similar to RS6 HUMAN 40S ribosomal protein S6 (Phosphoprotein NP33) [H.sapiens]
828	GCCGTGTCCG	350166	ribosomal protein S6
829	CCCATCCGAA	91379	ribosomal protein L26
830	CCCATCCGAA	356175	ESTs, Weakly similar to T46057 60S RIBOSOMAL PROTEIN-like
831	CCCGAGGCAG	45057	Homo sapiens, Similar to doublecortin and CaM kinase-like 1, clone MGC:45428 IMAGE:5532881, mRNA, complete cds
832	CCCGAGGCAG	155223	stanniocalcin 2
833	CCTGAAATTT	77492	heterogeneous nuclear ribonucleoprotein A0
834	CCTGAAATTT	12102	sorting nexin 3
835	CTCACTTTTT	9585	Homo sapiens cDNA FLJ30010 fis, clone 3NB692000154
836	CTCACTTTTT	76722	CCAAT/enhancer binding protein (C/EBP), delta

Table 3. Genes employed for the clustering analysis shown in Fig. 3B

SEQ ID NO:	Tag	Unigene	Gene name
837	GCTGTTGCGC	8102	ribosomal protein S20
838	TCCCCGTACA		
839	CACAAACGGT	195453	ribosomal protein S27 (metallopanstimulin 1)
840	CACAAACGGT	356178	ESTs, Moderately similar to T47903 ribosomal protein S27
841	CCCTGATTTT	183684	eukaryotic translation initiation factor 4 gamma, 2
842	CCCTGATTTT	1799	CD1D antigen, d polypeptide
843	TGGGCAAAGC	2186	eukaryotic translation elongation factor 1 gamma
844	TAACTTGTGA	295726	integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)
845	AGCACCTCCA	75309	eukaryotic translation elongation factor 2
846	GAGGGAGTTT	76064	ribosomal protein L27a
847	GAGGGAGTTT	356342	ESTs, Highly similar to 2113200C ribosomal protein L27a [Homo sapiens] [H.sapiens]
848	GCGACAGCTC	184582	ribosomal protein L24
849	CGCCGCCGGC	182825	ribosomal protein L35
850	GGCAAGCCCC	334895	ribosomal protein L10a
851	GGCAAGCCCC	187577	SRY (sex determining region Y)-box 21
852	AGCTCTCCCT	82202	ribosomal protein L17
853	AGCTCTCCCT	374588	ESTs, Highly similar to R5HU22 ribosomal protein L17, cytosolic
854	CGCTGGTTCC	179943	ribosomal protein L11
855	CGCTGGTTCC	289019	latent transforming growth factor beta binding protein 3
856	GAAACCGAGG	268053	R3H domain (binds single-stranded nucleic acids) containing
857	GAAACCGAGG	279813	hypothetical protein HSPC014
858	GAGGTCCCTG	374499	ESTs, Weakly similar to PS62 ARATH Proteasome subunit alpha type 6-2 (20S proteasome alpha subunit A2) [A.thaliana]
859	GAGGTCCCTG	74077	proteasome (prosome, macropain) subunit, alpha type, 6
860	TGAAATAAAA	9614	nucleophosmin (nucleolar phosphoprotein B23, numatrin)
861	TGAAATAAAA	48516	ESTs
862	CCCCAGCCAG	252259	ribosomal protein S3
863	CCCCAGCCAG	334861	hypothetical protein FLJ23059
864	TAAATAATTT	1197	heat shock 10kD protein 1 (chaperonin 10)
865	ATAATTCTTT	288806	Homo sapiens cDNA FLJ11778 fis, clone HEMBA1005911
866	ATAATTCTTT	539	ribosomal protein S29
867	TTAAACCTCA	170311	heterogeneous nuclear ribonucleoprotein D-like
868	TTAAACCTCA	347810	ESTs
869	GCCGAGGAAG	339696	ribosomal protein S12
870	GCCGAGGAAG	143067	KIAA1602 protein
871	GCCTGTATGA	180450	ribosomal protein S24
872	GCCTGTATGA	356794	ESTs, Weakly similar to RS24 ARATH 40S ribosomal protein S24 [A.thaliana]
873	GTGTTAACCA	74267	ribosomal protein L15
874	CTTCGAAACT	51299	NADH dehydrogenase (ubiquinone) flavoprotein 2 (24kD)
875	AAGGTCGAGC	184582	ribosomal protein L24
876	AAGGTCGAGC	356004	ESTs, Weakly similar to T47559 60S ribosomal protein-like
877	CTTTGGAAAT	6820	cyclin fold protein 1
878	CTTTGGAAAT	184222	Down syndrome critical region gene 1
879	CCCCCTGGAT	275243	S100 calcium binding protein A6 (calcyclin)
880	CGCCGGAACA	356448	ESTs, Weakly similar to RL4B ARATH 60S ribosomal protein L4-B (L1) [A.thaliana]
881	CGCCGGAACA	286	ribosomal protein L4
882	GTGTTGCACA	301251	Homo sapiens cDNA FLJ12014 fis, clone HEMBB1001685
883	GTGTTGCACA	165590	ribosomal protein S13
884	CAACTTAGTT	180224	myosin regulatory light chain
885	GGGGCAGGGC	9383	cysteine-rich with EGF-like domains 1
886	CCAAGTTTTT	75914	coated vesicle membrane protein
887	TTGGCAGCCC	76064	ribosomal protein L27a
888	GTTAACGTCC	178391	ribosomal protein L36a
889	GTTAACGTCC	355599	ESTs, Moderately similar to putative ribosomal protein [Arabidopsis thaliana] [A.thaliana]
890	GGAAGTTTCG	55847	mitochondrial ribosomal protein L51
891	CCCGTCCGGA	180842	ribosomal protein L13

Table 3. Genes employed for the clustering analysis shown in Fig. 3B

SEQ ID NO:	Tag	Unigene	Gene name
892	CCCGTCCGGA	356148	ESTs, Weakly similar to 60S ribosomal protein L13 [Arabidopsis thaliana] [A.thaliana]
893	GGCCGCGTTC	5174	ribosomal protein S17
894	GGCCGCGTTC	356626	Homo sapiens cDNA FLJ34449 fis, clone HLUNG2002145
895	AAAAGAAACT	172182	poly(A) binding protein, cytoplasmic 1
896	AAAAGAAACT	354497	ESTs
897	AACTCCCAGT	110571	growth arrest and DNA-damage-inducible, beta
898	AACTCCCAGT	118126	protective protein for beta-galactosidase (galactosialidosis)
899	CACTTTTGGG	321497	Homo sapiens cDNA FLJ31347 fis, clone MESAN2000023
900	CACTTTTGGG	334851	LIM and SH3 protein 1
901	GGGAGGGAAG	75243	bromodomain containing 2
902	GGGAGGGAAG	160953	p53-regulated apoptosis-inducing protein 1
903	GGGGGAATTT	129548	heterogeneous nuclear ribonucleoprotein K
904	CATCTAAACT	180900	Williams-Beuren syndrome chromosome region 1
905	TCCCCGTGGC	75616	24-dehydrocholesterol reductase
906	TCCCCGTGGC	356547	hypothetical protein BC016005
907	GCCTGCAGTC	31439	serine protease inhibitor, Kunitz type, 2
908	GCCTGCAGTC	273385	GNAS complex locus
909	AGAATTTGCA	250655	prothymosin, alpha (gene sequence 28)
910	AGAATTTGCA	374658	ESTs, Highly similar to TNHUA prothymosin alpha
911	TCGGAGCTGT	4055	Homo sapiens mRNA; cDNA DKFZp564C2063 (from clone DKFZp564C2063)
912	CACACAGTTT	204354	ras homolog gene family, member B
913	GTAATCCTGC		
914	AGAGGTGTAG		
915	TTAGCCAGGC	71367	similar to RIKEN cDNA 1110058L19
916	TTAGCCAGGC	161640	tyrosine aminotransferase
917	TGGAAAGTGA	25647	v-fos FBJ murine osteosarcoma viral oncogene homolog
918	TGGAAAGTGA	101047	transcription factor 3 (E2A immunoglobulin enhancer binding factors E12/E47)
919	TCCCTATTAA		
920	AGGAGCGGGG	252189	syndecan 4 (amphiglycan, ryudocan)
921	GCCCCTCCGG	83753	small nuclear ribonucleoprotein polypeptides B and B1
922	GCCCCTCCGG	180859	16.7Kd protein
923	GCTGCCCTTG	348557	tubulin alpha 6
924	GCTGCCCTTG	272897	tubulin, alpha 3
925	CCACCCCGAA	74637	testis enhanced gene transcript (BAX inhibitor 1)
926	GCTGCGGTCC	795	H2A histone family, member O
927	GCTGCGGTCC	106061	RD RNA-binding protein
928	GAGATCCGCA	75348	proteasome (prosome, macropain) activator subunit 1 (PA28 alpha)
929	CAGAGATGAA	8997	Sad1 unc-84 domain protein 1
930	GCAAGCCAAC		
931	TGGCCTGCCC	181002	MLL septin-like fusion
932	GCGGGGTGGA	85155	zinc finger protein 36, C3H type-like 1
933	AGGTGGCAAG		
934	TCGAAGCCCC	198281	pyruvate kinase, muscle
935	TTTAACGGCC		
936	ACTTTCCAAA	78921	A kinase (PRKA) anchor protein 1
937	TGGAAGCACT	624	interleukin 8
938	GTCCGAGTGC	351316	transmembrane 4 superfamily member 1
939	TAACAGCCAG	81328	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
940	TAACAGCCAG	235498	hypothetical protein FLJ14075
941	GCCTTGGGTG	2250	leukemia inhibitory factor (cholinergic differentiation factor)
942	TTTGAAATGA	28491	spermidine/spermine N1-acetyltransferase
943	GGGTAGGGGG	13323	hypothetical protein FLJ22059
944	ATCGTGGCGG	5372	claudin 4
945	ATCGTGGCGG	8026	sestrin 2
946	CCTGGCCTAA	297285	ESTs, Weakly similar to ZF37 HUMAN Zinc finger protein ZFP-37 [H.sapiens]
947	CCTGGCCTAA	111676	protein kinase H11

Table 3. Genes employed for the clustering analysis shown in Fig. 3B

SEQ ID NO:	Tag	Unigene	Gene name
948	AAGATTGGTG	1244	CD9 antigen (p24)
949	AATCCTGTGG	43910	CD164 antigen, sialomucin
950	AATCCTGTGG	178551	ribosomal protein L8
951	TGGTGTTGAG	275865	ribosomal protein S18
952	TGGTGTTGAG	374510	ESTs, Highly similar to S30393 ribosomal protein S18, cytosolic
953	CTGGCCCTCG	350470	trefoil factor 1 (breast cancer, estrogen-inducible sequence expressed in)
954	CTGGCCCTCG	43654	ceroid-lipofuscinosis, neuronal 6, late infantile, variant
955	GACTCTTCAG	234726	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3
956	CTGCCAACTT	180370	cofilin 1 (non-muscle)
957	GTGCGCTGAG	181244	major histocompatibility complex, class I, A
958	GTGCGCTGAG	277477	major histocompatibility complex, class I, C
959	TTGGGGTTTC	62954	ferritin, heavy polypeptide 1
960	TTGGGGTTTC	374602	ESTs, Weakly similar to putative ferritin [Arabidopsis thaliana] [A.thaliana]
961	GGAGGGGGCT	77886	lamin A/C
962	GGAGGGGGCT	110642	neurotensin receptor 1 (high affinity)
963	TTAGTTTTTA	323949	kangai 1 (suppression of tumorigenicity 6, prostate; CD82 antigen (R2 leukocyte antigen, antigen detected by monoclonal and antibody IA4))
964	TTAGTTTTTA	274404	plasminogen activator, tissue
965	CCCAAGCTAG	76067	heat shock 27kD protein 1
966	CCCAAGCTAG	374617	ESTs, Highly similar to HHHU27 heat shock protein 27
967	GTGCACTGAG	181244	major histocompatibility complex, class I, A
968	GTGCACTGAG	277477	major histocompatibility complex, class I, C
969	CAGACTTTTT	293884	helicase/primase complex protein
970	CAGACTTTTT	78683	ubiquitin specific protease 7 (herpes virus-associated)
971	AAAACATTCT	323562	hypothetical protein DKFZp564K142 similar to implantation-associated protein
972	CACCTAATTG		
973	GGGACGAGTG		
974	CAAGCATCCC		
975	AGCAGATCAG	119301	S100 calcium binding protein A10 (annexin II ligand, calpactin I, light polypeptide (p11))
976	AGCCCTACAA	95243	transcription elongation factor A (SII)-like 1
977	TGAAGTAACA	150580	putative translation initiation factor
978	GCTAGGTTTA		
979	CAAAATCAGG	79933	cyclin I
980	GGCTGGGGGC	75721	profilin 1
981	GGCTGGGGGC	352407	chromosome 1 amplified sequence 3
982	GGCCCTAGGC	78909	zinc finger protein 36, C3H type-like 2
983	GCTGAACGCG	99029	CCAAT/enhancer binding protein (C/EBP), beta
984	AAGAGCGCCG	8997	Sad1 unc-84 domain protein 1
985	AAGAGCGCCG	274402	heat shock 70kD protein 1B
986	AGGGTGAAAC	77608	splicing factor, arginine/serine-rich 9
987	AGGGTGAAAC	363356	EST
988	GATCCCAACT	118786	metallothionein 2A
989	GCCTACCCGA	23582	tumor-associated calcium signal transducer 2
990	CCAGGAGGAA	276	farnesyltransferase, CAAX box, beta
991	CCAGGAGGAA	180414	heat shock 70kD protein 8
992	CCAGTGGCCC	180920	ribosomal protein S9
993	CCAGTGGCCC	356713	ESTs, Moderately similar to T49955 40S ribosomal protein-like
994	GAAGCTTTGC	289088	heat shock 90kD protein 1, alpha
995	GAAGCTTTGC	356532	ESTs, Moderately similar to 1908431A heat shock protein HSP81-1 [Arabidopsis thaliana] [A.thaliana]
996	TGTGTTGAGA	181165	eukaryotic translation elongation factor 1 alpha 1
997	TGTGTTGAGA	356428	Homo sapiens mRNA expressed only in placental villi, clone SMAP83
998	GTGACAGAAG	129673	eukaryotic translation initiation factor 4A, isoform 1
999	GTGACAGAAG	356129	ESTs, Weakly similar to JC1453 translation initiation factor eIF-4A2
1000	CCTCGGAAAA	2017	ribosomal protein L38
1001	CCTCGGAAAA	343481	ESTs, Weakly similar to RL38 ARATH 60S ribosomal protein L38 [A.thaliana]
1002	CTCATAAGGA		

Table 3. Genes employed for the clustering analysis shown in Fig. 3B

SEQ ID NO:	Tag	Unigene	Gene name
1003	CTAGCCTCAC	14376	actin, gamma 1
1004	GGGCCAACCC	119475	cold inducible RNA binding protein
1005	GGGCCAACCC	226795	glutathione S-transferase pi
1006	ACCCCCCCGC	2780	jun D proto-oncogene
1007	GGTGCCCAGT	75607	myristoylated alanine-rich protein kinase C substrate
1008	GCTTTATTTG	288061	actin, beta
1009	GGCTCCCACT	74335	heat shock 90kD protein 1, beta
1010	CTAAGACTTC		
1011	GGGTAGCTGG		
1012	ACCCACGTCA	298184	potassium voltage-gated channel, shaker-related subfamily, beta member 2
1013	ACCCACGTCA	198951	jun B proto-oncogene
1014	GGGCAGGCGT	737	immediate early protein
1015	GTTCACTGCA	77318	platelet-activating factor acetylhydrolase, isoform Ib, alpha subunit (45kD)
1016	GTTCACTGCA	168383	intercellular adhesion molecule 1 (CD54), human rhinovirus receptor
1017	ACTCAGCCCG	101382	tumor necrosis factor, alpha-induced protein 2
1018	ACTCAGCCCG	4990	KIAA1089 protein
1019	TGATTTCACT		
1020	AGGTTTCCTC	9736	proteasome (prosome, macropain) 26S subunit, non-ATPase, 3
1021	ACCATCCTGC	32963	cadherin 6, type 2, K-cadherin (fetal kidney)
1022	ACCATCCTGC	76095	immediate early response 3
1023	GGGAGGTAGC	171825	basic helix-loop-helix domain containing, class B, 2
1024	CCGTCCAAGG	80617	ribosomal protein S16
1025	CTCACCGCCC	183650	cellular retinoic acid binding protein 2
1026	CCCGCCCCCG	155048	Lutheran blood group (Auberger b antigen included)
1027	ACTAACACCC		
1028	CACTACTCAC		
1029	CAGGAGGAGT	289101	glucose regulated protein, 58kD
1030	CAGGAGGAGT	356023	ESTs, Weakly similar to PDI2 ARATH Probable protein disulfide isomerase 2 precursor (PDI) [A.thaliana]
1031	GCGACCGTCA	273415	aldolase A, fructose-bisphosphate
1032	AAGGGAGGGT	182248	sequestosome 1
1033	GGCAGCCAGA	75061	macrophage myristoylated alanine-rich C kinase substrate
1034	GGCAGCCAGA	144501	ESTs
1035	TGTGGGTGCT	306339	Homo sapiens mRNA; cDNA DKFZp586N2022 (from clone DKFZp586N2022)
1036	TGTGGGTGCT	194657	cadherin 1, type 1, E-cadherin (epithelial)
1037	ATTTGAGAAG	178658	RAD23 homolog B (S. cerevisiae)
1038	AATGGAAATC	4943	melanoma antigen, family D, 2
1039	AATGGAAATC	58103	A kinase (PRKA) anchor protein (yotiao) 9
1040	TTTGGGCCTA	17409	cystein rich protein (CRP1)
1041	CAACTAATTC	69997	zinc finger protein 238
1042	CAACTAATTC	75106	clusterin (complement lysis inhibitor, SP-40,40, sulfated glycoprotein 2, testosterone-repressed prostate message 2, apolipoprotein J)
1043	GTTGTGGTTA	75415	beta-2-microglobulin
1044	GTTGTGGTTA	99785	Homo sapiens cDNA: FLJ21245 fis, clone COL01184
1045	TTAAATGGAA	33944	ESTs, Weakly similar to hypothetical protein FLJ20489 [Homo sapiens] [H.sapiens]
1046	TTAAATGGAA	351593	fibrinogen, A alpha polypeptide
1047	CTTAAAAAAA	306309	Homo sapiens mRNA; cDNA DKFZp566L0824 (from clone DKFZp566L0824)
1048	CTTAAAAAAA	75063	human immunodeficiency virus type I enhancer binding protein 2
1049	CTTCTCCAAA	151242	serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1, (angioedema, hereditary)
1050	CTTCTCCAAA	6671	COP9 constitutive photomorphogenic homolog subunit 4 (Arabidopsis)
1051	TACCTGCAGA	100000	S100 calcium binding protein A8 (calgranulin A)
1052	ATAATAAAAG	89690	GRO3 oncogene
1053	ATAATAAAAG	250879	Homo sapiens cDNA FLJ25968 fis, clone CBR01977
1054	AGAAAGATGT	352541	hypothetical protein MGC29937
1055	AGAAAGATGT	78225	annexin A1

Table 3. Genes employed for the clustering analysis shown in Fig. 3B

SEQ ID NO:	Tag	Unigene	Gene name
1056	GTGCGGAGGA	332053	serum amyloid A1
1057	GTGCGGAGGA	336462	serum amyloid A2
1058	GGAAAAGTGG	265317	hypothetical protein MGC2562
1059	GGAAAAGTGG	297681	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1
1060	AATAGGTCCA	113029	ribosomal protein S25
1061	AATAGGTCCA	356801	ESTs, Weakly similar to T08568 ribosomal protein S25, cytosolic
1062	GTTTATGGAT	365706	matrix Gla protein
1063	CAACAATAAT	283683	chromosome 8 open reading frame 4
1064	TTTATTTTAA	46452	secretoglobin, family 2A, member 2
1065	CTTCCTGTGA	348419	small breast epithelial mucin
1066	TAAAAACTTT	204096	secretoglobin, family 1D, member 2
1067	TAAAAACTTT	343411	Homo sapiens mRNA; cDNA DKFZp586K2322 (from clone DKFZp586K2322)
1068	ACACAGCAAG	27115	ESTs, Weakly similar to SFRB HUMAN Splicing factor arginine/serine-rich 11 (Arginine-rich 54 kDa nuclear protein) (P54) [H.sapiens]
1069	TGCAGCACGA	277477	major histocompatibility complex, class I, C
1070	TGCAGCACGA	110309	major histocompatibility complex, class I, F
1071	ACTCCAAAAA	356465	ESTs, Moderately similar to S71259 ribosomal protein S15, cytosolic
1072	ACTCCAAAAA	344078	Homo sapiens, clone IMAGE:3840457, mRNA
1073	GCCTCCTCCC	283781	muscle specific gene
1074	GCCTCCTCCC	319084	EST
1075	AAGCTCGCCG	62492	secretoglobin, family 3A, member 1, HIN-1
1076	CCTGGTCCCA	23881	keratin 7
1077	CCTGGTCCCA	167679	SH3-domain binding protein 2
1078	GAATTAACAT	79474	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide
1079	GAATTAACAT	90073	CSE1 chromosome segregation 1-like (yeast)
1080	TAATTTGCGT	79368	epithelial membrane protein 1
1081	TTGGTTTTTG	164021	small inducible cytokine subfamily B (Cys-X-Cys), member 6 (granulocyte chemotactic protein 2)
1082	TTGGTTTTTG	170088	SLC2A4 regulator
1083	GCTTGCAAAA	6823	neuropilin (NRP) and tolloid (TLL)-like 2
1084	GCTTGCAAAA	372783	superoxide dismutase 2, mitochondrial
1085	GCCGCCCTGC	76394	enoyl Coenzyme A hydratase, short chain, 1, mitochondrial
1086	GCCGCCCTGC	82208	acyl-Coenzyme A dehydrogenase, very long chain
1087	CTTCCAGCTA	217493	annexin A2
1088	CTTCCAGCTA	101651	Homo sapiens mRNA; cDNA DKFZp434C107 (from clone DKFZp434C107)
1089	CGAATGTCCT	335952	keratin 6B
1090	TTGAAACTTT	789	GRO1 oncogene (melanoma growth stimulating activity, alpha)
1091	TTGAAGCTTT	302738	Homo sapiens cDNA: FLJ21425 fis, clone COL04162
1092	CCCGGGAGCG	75807	PDZ and LIM domain 1 (elfin)
1093	CCCGGGAGCG	273186	chaperone, ABC1 activity of bc1 complex like (S. pombe)
1094	GGACTCTGGA	71	alpha-2-glycoprotein 1, zinc
1095	GGACTCTGGA	56023	brain-derived neurotrophic factor
1096	GTCTTAAAGT	177781	Homo sapiens, clone IMAGE:4711494, mRNA
1097	CAGCTCACTG	738	ribosomal protein L14
1098	CAGCTCACTG	356012	ESTs, Weakly similar to T06039 ribosomal protein L14 homolog T24A18.40

Example 3. Molecular Markers in DCIS

To determine if there are genes that are statistically significantly more likely to be expressed in DCIS than in invasive tumors (and vice versa), various statistical tests were performed (see Example 1). Based on these analyses, the levels of expression of CD74 and a SAGE tag (CTGGGCGCCC) (SEQ ID NO:1109) with no database match were found to be significantly greater in invasive or metastatic tumors than in DCIS ($p=0.02$ and $p=0.05$, respectively, Table 4). The samples studied were the same as those shown in Table 1; the sample designated "M1" in Table 4 was the same as that designated "MET" in Table 1. The expression of MGC2328, IBC-1, and eight other genes was also more likely to occur in invasive/metastatic tumors than in DCIS, but none of these differences in expression reached statistical significance (Table 4). Similarly the expression of S100A7 and keratin 19 ("KRT19") was more frequent and at higher levels in DCIS than in invasive/metastatic tumors but this difference in expression was only marginally statistically significant.

In a second statistical analysis, ROC (receiver operating characteristic) curve analysis was used to choose the "best cut-off" for values, i.e., the cut-off that results in the most samples being correctly classified as DCIS or invasive, weighing both kinds of misclassification equally (Table 4). Tags that do not include 0.50 in the confidence interval (CI) could be useful for the differential diagnosis of *in situ* versus invasive carcinomas. Such tags include all those with $p \leq 0.13$ using the higher of two normals' cut-off as well as 3 other high in DCIS tags and 3 other high in invasive tags (Table 4). Using the best cut-off values, several of the SAGE tags correctly classified most of the DCIS and invasive SAGE libraries. For example KRT19 expression classified 75% of the DCIS and 0% of the invasive libraries as DCIS, while MGC23280 expression diagnosed 78% of the invasive cancer and 0% of the DCIS libraries as "invasive". Thus, MGC23280 expression had 78% sensitivity and 100% specificity to correctly categorize breast tumors as DCIS or invasive/metastatic in this data set.

Table 4. Genes specific for in situ and invasive or metastatic breast cancer SAGE libraries

SEQ ID NO:	Tag sequence	Unigene	Gene	P-value	ROC area x100	ROC area x100 95% CI	ROC best cut-off	DCIS % >cut-off	IDC % > cut-off	N1	N2	D1	D2	D3	D4	D5	D6	D7	T18	I1	I2	I3	I4	I5	I6	LN1	LN2	M1
	DCIS specific genes																											
1099	GAGCAGCGCC	112408	S100A7* (psoriasin)	0.29	92	77-100	2.00	88	11	18	0	1018	3	3	373	16	1	2	890	0	0	0	1	0	20	0	0	0
1100	GCTCTGCTTG	112408	S100A7* (psoriasin)	0.08	69	51-87	54.70	38	0	2	0	76	0	0	20	0	0	0	55	0	0	0	0	0	0	0	0	0
1101	GGACCTTTAT	352107	TFF3* (trefoil factor 3)	0.33	64	35-93	3.00	50	11	2	0	23	3	0	1	23	1	0	37	2	1	1	0	1	0	4	3	0
1102	CTCCACCCGA	352107	TFF3* (trefoil factor 3)	1.00	69	42-97	16.80	100	56	34	7	511	854	17	26	451	31	38	261	369	124	15	0	94	16	285	244	2
1103	GTGGCCACGG	112405	S100A9 (calgranulin B)	0.29	85	63-100	4.10	88	22	29	30	200	0	9	238	4	20	15	92	0	1	1	3	0	72	0	0	4
1104	GACATCAAGT	182265	KRT19 (keratin 19)	0.06	83	58-100	58.90	75	0	33	35	59	165	3	118	139	59	153	34	20	40	41	25	31	20	10	34	16
1105	CCCTACCCTG	75736	APOD (apolipoprotein D)	0.21	76	52-100	7.70	100	44	4	58	15	42	8	293	215	9	12	49	2	16	41	3	4	44	0	3	16
	Invasive or metastatic breast cancer specific genes																											
1106	ACGTTAAAGA	350570	IBC-1 (Invasive Breast Cancer-1)	0.13	75	55-95	2.50	0	56	0	0	0	0	0	1	0	0	0	0	177	101	3	0	0	12	199	0	0
1107	CCAGAGAGTG	180884	CPB1 (carboxypeptidase B1)	0.33	67	43-91	1.30	25	56	0	0	0	9	0	0	0	0	21	0	107	115	0	1	0	0	0	354	2
1108	GGAGTAAGGG	5163	MGC23280 (hypothetical protein)	0.06	86	68-100	1.46	0	78	0	0	0	0	0	1	0	0	1	0	22	8	0	3	1	0	22	1	2
1109	CTGGGCGCCC	NA	No reliable match	0.05	80	61-99	12.00	0	56	0	0	0	0	2	0	0	0	0	0	40	25	0	0	0	12	26	1	34
1110	CCAATAAAGT	101850	RBP1 (retinol binding protein)	0.33	78	54-100	6.40	25	78	2	0	0	3	0	0	2	6	11	7	49	28	6	8	0	0	102	32	21
1111	TTTGTTTTTA	131740	FLJ30428 (hypothetical protein)	1.00	84	62-100	4.01	0	78	0	0	0	3	2	3	2	1	4	2	7	7	27	4	21	4	2	18	0
1112	ATCCGCGAGG	180142	CLSP (calmodulin-like skin protein)	0.64	64	38-89	19.00	25	56	0	0	0	0	3	22	0	20	0	0	47	25	0	52	19	0	20	0	0
1113	GACCACACCG	367741	NUDT8 (nudix)	0.64	69	43-96	8.00	0	56	2	2	2	0	0	7	0	7	0	5	27	21	1	0	0	8	33	9	0
1114	CGATATTCCC	37616	MGC14480 (hypothetical protein)	0.33	79	57-100	6.40	25	78	4	2	4	6	0	3	12	1	6	7	36	26	6	4	9	12	31	13	2
1115	AAACCCCAAT	181125	IGL (immunoglobulin lambda)	1.00	72	46-97	38.00	25	67	0	0	15	0	17	102	4	1	1	44	163	87	78	3	0	241	258	10	38
1116	GTTCACATTA	84298	CD74 antigen	0.02	93	81-100	31.70	25	100	7	33	29	6	25	188	70	6	13	28	159	208	226	32	428	474	203	72	72

*From two transcripts (S100A7 and TFF3) two independent SAGE tags were derived and both found to be specific for DCIS.

P-value is based on using the SAGE tag number which was highest of two normals as cut-off.

The first ROC column gives the ROC area, the second the approximate 95% CI, the third column gives the "best" cut-off, while the last two columns show the percent of DCIS specimens with values greater than or equal to the ROC best cut-off and the percent of invasive specimens with values greater than or equal to the ROC best cut-off.

Next, 26 genes that appeared to be the most highly differentially expressed between normal and DCIS samples or between intermediate (D2) and high-grade (D1) DCIS at $p \leq 0.001$ using the SAGE 2000 software were selected for further validation studies (Table 5). It was hypothesized that genes most highly differentially expressed between normal and DCIS tissue or two different types of DCIS tumors could be used as molecular markers for defining biologically and potentially clinically meaningful subgroups of DCIS. This concept was supported by the observation that clustering analysis of the eight DCIS libraries using only these 26 genes gave a dendogram (Fig. 3C) that was almost identical to that obtained using 582 genes (Fig. 3B). In Table 5, the samples shown are the same as those shown in Table 4 and the column labeled "Method" indicates the technique used to validate the conclusions of the relevant SAGE data (ISH, in situ hybridization; IH, immunohistochemistry; ND, not done).

Table 5. Genes selected for mRNA in situ hybridization and immunohistochemical analyses

SEQ ID:	Tag_Sequence	Unigene	Gene	N1	N2	D1	D2	D3	D4	D5	D6	D7	T18	I1	I2	I3	I4	I5	I6	LN1	LN2	M1	Method
	"Normal specific"																						
1117	AAGCTCGCCG	62492	SCGB3A1 (HIN-1, High in Normal-1)	125	44	0	0	0	3	0	9	0	0	0	0	0	0	0	0	0	0	4	ISH
1118	GTCCGAGTGC	351316	TM4SF1 (transmembrane 4 superfamily member 1)	134	96	11	33	11	1	2	23	13	4	2	0	0	8	0	8	2	3	5	ISH
1119	GACTGCGCGT	10086	FN14 (Type I transmembrane protein Fn14)	40	26	0	36	6	3	4	22	32	4	0	3	0	1	1	8	0	0	0	ND
1120	TTGAAGCTTT	75765	CXCL2 (GRO2, growth related protein 2)	122	247	2	3	15	0	0	29	5	0	0	1	4	0	0	0	0	0	0	IH
1121	TTGAAACTTT	789	CXCL1 (GRO1, growth related protein 1)	394	453	11	12	14	1	0	61	1	4	0	0	1	0	1	0	0	0	2	IH
1122	TGGAAGCACT	624	IL-8 (interleukin-8)	368	352	8	39	12	1	0	94	15	0	2	0	1	0	0	0	0	0	0	IH
1123	TAACAGCCAG	81328	NFKBIA (NFKB inhibitor alpha)	136	152	6	39	23	4	2	28	125	19	4	7	8	7	9	4	2	10	20	IH
	"Tumor specific"																						
1124	CAATTAAAAG	149923	XBP1 (X-box binding protein)	80	58	147	196	29	366	322	27	97	214	244	247	535	18	531	129	199	599	7	ISH
1125	TTTGGTGTTT	83190	FASN (fatty acid synthase)	5	0	8	24	2	57	27	5	28	21	36	41	62	14	57	12	28	10	4	IH
1126	TGATCTCCAA	83190	FASN (fatty acid synthase)	16	5	53	63	6	201	182	31	47	5	168	33	105	17	314	4	254	46	21	IH
1127	CTCCACCCGA	82961	TFF3 (trefoil factor 3)	34	7	511	854	17	26	451	31	38	261	369	124	15	0	94	16	285	244	2	ISH+IH
	"Intermediate-grade DCIS specific"																						
1128	CGCCGACGAT	265827	IFI-6-16 (interferon alpha-uinducible protein)	4	0	17	644	3	90	418	18	366	4	130	171	5	63	12	161	14	526	181	ISH
1129	TTTGGGCCTA	17409	CRIP1 (cyteine-rich protein 1)	33	5	21	66	29	22	33	49	223	4	7	49	37	0	35	4	2	60	7	ISH
1130	AATCTGCGCC	833	ISG15 (interferon-stimulated protein, 15 kDa)	0	0	2	48	2	3	20	1	42	2	9	5	1	0	1	28	4	29	16	ISH
1131	CCAGGGGAGA	278613	IFI27 (interferon alpha inducible protein)	0	0	4	36	3	4	90	5	176	2	0	21	5	1	3	104	2	31	77	ISH
1132	GAAAGATGCT	334370	BEX1 (brain expressed, X-linked 1)	2	0	6	48	0	1	0	1	1	0	29	37	1	1	1	0	0	162	2	ISH
1133	CAGACTTTTT	293884	LOC150678 (helicase/primase protein)	7	5	4	54	5	1	4	0	31	5	2	9	4	1	4	0	0	4	4	ISH
1134	CTGGCGCCGA	183180	ANAPC11 (anaphase promoting complex subunit 11)	4	2	11	42	2	7	29	2	2	12	22	17	19	11	15	28	26	28	20	ND
1135	TGAGCTACCC	72222	*FER1L4 (Fer-1-like 4)*	0	0	0	33	0	0	6	0	0	11	2	0	0	1	0	4	0	0	0	ND
	"High-grade DCIS specific"																						
1136	GAGCAGCGCC	112408	S100A7 (psoriasin)	18	0	1018	3	3	373	16	1	2	890	0	0	0	1	0	20	0	0	0	ISH+IH
1137	TTTGCACCTT	75511	CTGF (connective tissue growth factor)	0	0	141	6	18	63	18	9	6	41	9	42	43	66	19	16	10	7	48	ISH+IH
1138	TATGAGGGTA	24950	RGS5 (regulator of G-protein signaling 5)	0	0	40	0	0	1	0	0	6	46	4	0	1	0	0	8	0	1	4	ISH
1139	GAAGTTATAA	137476	PEG10 (paternally expressed 10)	0	7	44	3	0	6	0	33	1	16	0	4	0	4	1	0	8	0	0	ISH
1140	ATGTGAAGAG	111779	SPARC (osteonectin)	4	0	118	3	6	79	39	22	6	12	112	97	185	47	194	96	163	32	129	IH
1141	GAGAGAAAAT	181444	LOC51235 (hypthetical protein)	0	2	40	9	0	10	6	7	7	21	4	8	9	11	18	0	6	10	27	ND
1142	CTCCCCCAAA	293441	SNC73 (immunoglobulin heavy mu chain)*	2	14	78	0	20	605	37	1	0	11	159	86	186	0	6	12	140	19	109	ISH

ISH=in situ hybridization, IH=immunohistochemistry, ND= not determined.

* The expression of SNC73 was found to be localized to leukocytes and was not pursued further.

Example 4. Confirmation of SAGE Gene Expression Studies by mRNA *in situ* Hybridization

mRNA *in situ* hybridization determines gene expression at the cellular level and is particularly useful in solid tumors that are heterogeneous in cellular composition. Eighteen frozen DCIS and invasive breast cancer samples were used for such a study. Whenever possible tumors were selected to include normal, DCIS, and invasive components on the same slide in order to obtain expression data in these three stages of breast tumorigenesis. Examples of *in situ* hybridization results are depicted in Fig. 4A. Interestingly, the upregulation in expression of several genes in DCIS occurred mostly, or exclusively, in non-epithelial cells. Specifically, CTGF (Connective Tissue Growth Factor) and RGS5 (Regulator of G protein Signaling) were highly expressed in DCIS myoepithelial cells and stromal fibroblasts; in certain tumors expression was upregulated in DCIS epithelial cells as well (Fig. 4A). Cumulative scores for *in situ* hybridization were used for hierarchical clustering analysis and statistical tests. A dendogram of the 18 different tumors and 5 normal breast tissues showed that, using the expression of 14 genes, it was possible to distinguish between normal and cancer samples and group the tumors into subclasses (Fig. 4B). Although a clustering analysis of gene expression profiles obtained by *in situ* hybridization in DCIS of different grades contained some inconsistent associations, there was an indication that, as shown by the clustering analysis of DCIS tumors using SAGE data, DCIS tumors of a particular grade were more similar to each other with respect to the expression of the 14 genes than they were to DCIS tumors of a different grade (data not shown). The expression of no single gene was found to distinguish between DCIS and invasive tumors; this finding confirmed the results of the SAGE analysis described above. Surprisingly, in the majority of cases, the *in situ* and invasive areas within particular tumors did not always show the highest similarity to each other (Fig. 4B). This result is consistent with the idea that gene expression profiles are not the same during tumor progression.

Fisher's exact test revealed significant positive correlation between the expression of TFF3 and IFI-6-16 ($p=0.01$), LOC51235 and BEX1 ($p=0.05$), while inverse correlation was found between the expression of S100A7 and RGS5Tu ($p=0.04$), S100A7 and TFF3 ($p=0.04$), and CTGF and TM4SF1 ($p=0.01$). No statistically significant associations were found between the expression of any of these genes and histo-pathologic features of the tumors.

Example 5. Immunohistochemical Analysis of Gene Tissue Microarrays and Clinicopathologic Associations

The expression of 10 genes was analyzed by immunohistochemistry using tissue microarrays composed of tumors of different pathologic stages. In total, 788 tumor samples (675 primary invasive tumors, 33 metastases, 71 pure DCIS, and 9 DCIS with concurrent invasive carcinoma) obtained from eight different cohorts (tissue microarrays) were analyzed. Expression of all 10 genes was not analyzed in all cohorts. An example of immunohistochemical staining of a DCIS with antibodies specific for 5 gene products is depicted in Fig. 4C.

Cumulative scores for immunohistochemical staining were used for statistical analyses to determine associations between the expression of the genes and histo-pathologic features of the tumors or between different genes. In addition, S100A7 expression was analyzed with respect to clinical outcome (overall survival and distant metastasis free survival) in two of the patient cohorts.

As shown by the above-described SAGE analyses, the expression of IBC-1 was almost exclusively limited to a subset of invasive breast carcinomas, with only 2 out of 80 DCIS tumors showing detectable IBC-1 expression (Fig. 4C and data not shown). The expression of CTGF, TFF3, and SPARC in the stroma was statistically significantly related to pathologic stage with TFF3 and SPARC being less likely to be expressed in DCIS than in invasive or metastatic tumors (Table 6). Statistically significant association between S100A7 expression and estrogen receptor (ER) negativity, high histologic grade, and more than 4 positive lymph nodes was demonstrated in logistic regression models in primary invasive tumors (Table 6). Since all these tumor characteristics are known to correlate with poor prognosis, it is likely that S100A7 expression identifies a clinically meaningful subgroup of tumors. Kaplan-Meier analysis demonstrated decreased overall survival for patients with S1007 A7 positive tumors, but this did not reach statistical significance ($p=0.41$), possibly due to relatively short patient follow-up data and insufficient sample size (data not shown). The expression of fatty acid synthase (FASN) was higher in ER negative and HER2 positive high-grade tumors, while the expression of SPARC (osteonectin) inversely correlated with high histologic grade and TNM stage 3 (Table 6). The fraction of breast tumors that expressed the cytokines CXCL1 (GRO1), CXCL2 (GRO2), and IL-8 was, as expected, very low, since the genes encoding them were more highly expressed in normal mammary epithelium than in breast cancer assessed by SAGE and

immunohistochemistry (data not shown). Finally, using Fisher's exact test the expression of S100A7 was associated with a higher likelihood of expression of FASN ($p=9.95x10^{-6}$) and TFF3 ($p=0.002$), and a lower likelihood of expression of CTGF ($p=0.005$), while the expression of FASN was associated with that of TFF3 ($p=3.5x10^{-6}$) and SPARC in the tumor cells ($p=4x10^{-5}$).

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Table 6. Relationships between gene expression and histopathologic features of tumors

					DCIS				Invasive			
	DCIS	Invasive	Metastasis	#p-value	age ≤ 50	ER	HER2	Grade 1	Grade 3	Stage 3	Tumor size ≥ 4 pos LN	
S100A7	23 (37.5)	245 (43.4)	16 (31.4)	0.08	p=0.03	*p=0.03	NS	NS	p<0.0001	NS	NS	p=0.0008
FASN	28 (38.9)	126 (51.0)	21 (50.0)	0.2	NS	p=0.02	p=0.002	*p=0.03	NS	NS	NS	NS
TFF3	36 (52.2)	196 (77.2)	31 (75.6)	0.0003	NS	p=0.02	NS	NS	NS	NS	NS	NS
CTGF	21 (30.0)	88 (34.7)	5 (12.2)	0.01	NS	NS	NS	NS	NS	NS	NS	NS
SPARC-Tumor	27 (39.1)	136 (50.4)	21 (50.0)	0.25	NS	NS	NS	NS	*p=0.01	*p=0.02	NS	NS
SPARC-Stroma	63 (87.5)	248 (91.2)	42 (100.0)	0.04	NS	NS	NS	NS	NS	*p=0.002	p=0.03	NS
CXCL1 (GRO1)	ND	11 (15.9)	ND	NA	NA	NS	NS	NS	NS	NS	NS	NS
CXCL2 (GRO2)	ND	2 (3.1)	ND	NA	NA	NS	NS	NS	NS	NS	NS	NS
IL-8	ND	5 (7.5)	ND	NA	NA	NS	NS	NS	NS	NS	NS	NS
NFKBIA	ND	46 (93.9)	ND	NA	NA	NS	NS	NS	NS	NS	NS	NS
CCND1	ND	3 (10.7)	ND	NA	NA	NS	NS	NS	NS	NS	NS	NS
CD45	ND	28 (96.6)	ND	NA	NA	NS	NS	NS	NS	NS	NS	NS

Numbers reflect the actual numbers of tumor specimens that were positive for the indicated gene, and the % of positive tumors is indicated in parenthesis.
Only data for which there was at least one statistically significant association is listed in the table.
#p-value is Fisher's exact test p-value for association between gene expression and tumor category (DCIS, Invasive, or Metastasis).
All other p-values are likelihood ratio (LR) test p-values.
*denotes p-value for inverse correlation.

Example 6. Analysis of SAGE libraries from epithelial and non-epithelial cells of normal breast and DCIS tissue

The SAGE analyses described above indicated that, in breast cancer, dramatic changes occur not only in the cancerous epithelial cells, but also in various stromal cells. Surprisingly all these stromal changes were already present in pre-invasive tumors such as DCIS (ductal carcinoma in situ) that have not yet invaded the surrounding tissues. Interestingly, many of the genes up-regulated in tumor epithelial or stromal cells encode secreted proteins (Connective Tissue Growth Factor, Trefoil Factor 3, Osteonectin, IGFBP-7 etc.) implicating autocrine and/or paracrine regulatory loops among epithelial and stromal cells. Based on these results it was concluded that a comprehensive analysis of the gene expression profile of each cell type found in normal breast tissue and DCIS tissue, combined with the analysis of the genetic changes present in these cells would yield important new information on the role of epithelial-stromal interactions in breast tumorigenesis and will help define the cell type of origin of breast carcinomas. In addition, genes and pathways identified by such an approach will likely represent excellent candidate therapeutic targets.

Analysis of SAGE libraries from epithelial and non-epithelial cells from normal breast tissue and DCIS tumors identified 35 tags that are significantly ($p \leq 0.002$) differentially expressed between leukocytes (Table 7), 333 tags that are significantly ($p \leq 0.002$) differentially expressed between myoepithelial cells (Table 8), 146 tags that are significantly ($p \leq 0.002$) differentially expressed between luminal epithelial cells (Table 9), and 175 tags that are significantly ($p \leq 0.002$) differentially expressed between endothelial cells (Table 10) isolated from normal and two different DCIS tissue. In Tables 7-10, data obtained with normal breast tissue (NL) and one DCIS sample (Table 10: D6) or two DCIS samples (Tables 7-9: D6 and D7) are shown. The numbers of tags shown are normalized values (see Example 1). The ratio of the number of tags obtained from cells isolated from DCIS tissue to the number obtained with cells from normal breast tissue (d/n, d6/n, or d7/n) for each tag are shown. The tables also include the Unigene numbers and the names of previously identified genes. Where no Unigene number is shown, the relevant gene has not previously been identified.

Analysis of the SAGE data confirmed the findings of the RT-PCR analysis (see Example 1 and Figure 2) that the cell purification procedure worked well in that certain genes known to be expressed in the cell types of interest were represented in the relevant SAGE libraries. For

example, the leukocyte libraries had the highest level of expression of several immunoglobulin and certain interleukins, while the levels of IGFBP-7 and hevin, and selectin E (endothelial cell adhesion molecule) were highest in the endothelial cell SAGE libraries. Interestingly, keratin 7 and 17 were highly abundant in the normal, but significantly decreased in the DCIS myoepithelial libraries suggesting that maintaining the normal differentiation state of myoepithelial cells may require the presence of normal luminal mammary epithelial cells. In many of the genes, there was at least a 10-fold difference in expression between normal and one or both DCIS tissues tested; in Tables 7-10 the relevant genes are indicated by the symbol "d" at the end of the relevant tag sequence. Furthermore, at least among differentially expressed genes that were previously known, 44 in the endothelial, 11 in the leukocyte, 82 in the myoepithelial, and 29 in the luminal epithelial cells encode proteins that are either secreted or expressed on the cell surface and thus likely to be involved in epithelial-stromal cell interactions that regulate (up or down) tumor development and/or progression; Tables 11, 12, 13, and 14 list the relevant genes in leukocytes, myoepithelial cells, luminal epithelial cells, and endothelial cells, respectively.

Table 7. Genes differentially expressed in leukocytes from DCIS and normal breast tissue

	Tag_Sequence	SEQ ID NO:	NL	D6	D7	d/n	Unigene	Gene
1	ACAGCGCTGA d	1143	0	192	32	Infinite	375570	HLA-DRB1, major histocompatibility complex, class II, DR beta 1
2	CAATTTGTGT d	1144	0	44	32	Infinite	126256	interleukin 1, beta
3	GCCGGGTGGG d	1145	2	21	32	13	74631	basigin (OK blood group), leukocyte activation M6 antigen
4	CGACCCCACG d	1146	14	164	60	8	169401	apolipoprotein E
5	GCACCAAAGC d	1147	19	396	192	16	73817	small inducible cytokine A3
6	GAAATACAGT d	1148	6	128	69	16	67201	NT5C, 5',3'-nucleotidase, cytosolic
7	ACCGCCGTGG d	1149	4	29	50	10	68877	cytochrome b-245, alpha polypeptide-neutrophil specific
8	TCCCTGGCTG d	1150	2	31	28	14	78575	prosaposin, short alt. transcipt, 88% con. Match
9	GGGCATCTCT d	1151	37	810	243	14	76807	major histocompatibility complex, class II, DR alpha
10	ATCCGGACCC d	1152	2	33	32	16	76556	protein phosphatase 1, regulatory (inhibitor) subunit 15A-induced by dNA damaga, may be involved in apoptosis
11	TTTGGGCCTA d	1153	2	21	35	13	17409	cysteine-rich protein 1 (intestinal)
12	GCTTTATTTG d	1154	14	51	142	7	288061	actin, beta
13	TTCCCTTCTT d	1155	4	40	35	9	814	major histocompatibility complex, class II, DP beta 1
14	TCCAAATCGA d	1156	4	64	38	12	297753	vimentin
15	AACCACATTG d	1157	2	22	41	15	179657	plasminogen activator, urokinase receptor
16	GCGGTTGTGG d	1158	17	181	76	8	79356	Lysosomal-associated multispanning membrane protein-5, haematopoetic cell specific
17	AAGTTGCTAT	1159	6	37	54	7	78575	prosaposin (variant Gaucher disease and variant metachromatic leukodystrophy)
18	ATGTAAAAAA d	1160	2	148	35	44	337778	lysozyme (renal amyloidosis)-leukocyte spec
19	GTAGGGGTAA d	1161	77	7	16	0		no confident match
20	GGGCCAGGGG d	1162	37	7	3	0	111099	hypothetical protein MGC10974, some homology to collagen a
21	GGGGGACGGC d	1163	41	3	6	0	367663	cDNA FLJ37864 fis, clone BRSSN2015982, 86% conf. match; some homology to actinin
22	CTGTTGGTGA	1164	60	11	13	0	3463	40S RIBOSOMAL PROTEIN S23
23	TAAGGAGCTG d	1165	234	17	32	0	299465	RS26_HUMAN 40S RIBOSOMAL PROTEIN S26
24	ACAAAAACTA d	1166	48	5	6	0		mitochondrial
25	TGGCTAAAAA d	1167	35	4	3	0	T52757	EST, but only 77% confidence match
26	ACTTTTTAAA d	1168	66	3	6	0	BG21616	ESTs
27	TACAGAGGGA d	1169	29	4	0	0	3776	zinc finger protein 216
28	CTCCACCCGA d	1170	79	8	0	0	352107	trefoil factor 3 (intestinal)
29	AGCTGTCCCC d	1171	130	7	3	0		mitochondrial
30	TGAAGCAGTA d	1172	27	2	0	0	AA12959	EST
31	TAATAAAGAA d	1173	27	1	0	0	17893	keratin 15, potentail contaminating epithelial cells?
32	GTGCCCGTGC d	1174	27	1	0	0	356372	ESTs, Highly similar to TPIS_HUMAN TRIOSEPHOSPHATE ISOMERASE [H.sapiens]
33	CCCGCCTCTT d	1175	68	0	3	0		no confident match, tag highly abundant in some brain libs+kidney and norm colon, does not look Ly spec
34	ACACAGCAAG d	1176	358	0	6	0	AW57269	ESTs, 77% conf. match, tag high in organoids+norm breast epi-probably epi contaminant
35	GTCCCTGCCT d	1177	33	0	0	0	279837	GSTM2, glutathione S-transferase M2 (muscle)

Table 8. Genes differentially expressed in myoepithelial cells from DCIS and normal breast tissue

SEQ ID NO:	Tag_Sequence	NL	D6	D7	6/n	d7/n	Unigene	Gene
1178	ACCAAAAACC d	2	849	274	553	179	172928	collagen, type I, alpha 1, internally primed site
1179	TGGAAATGAC d	0	228	50	228	50	172928	collagen, type I, alpha 1, shorter alternative transcript
1180	CCACGGGATT d	0	185	55	185	55		No match
1181	GATCAGGCCA d	0	181	191	181	191	119571	Collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant, shorter alternative transcript
1182	TTTGGTTTTC d	0	154	24	154	24	179573	retinoblastoma binding protein 1, reliable 3'end.
1183	AACTCCCAGT d	3	351	427	114	139	110571	growth arrest and DNA damage inducible beta, reliable 3' end
1184	GACTTTGGAA d	0	110	36	110	36	172928	collagen, type I, alpha 1, internal tag
1185	CAACCAGTAA d	0	106	74	106	74	AA723001	zg89d05.s1 Soares_fetal_heart_NbHH19W Homo sapiens cDNA clone IMAGE:409737 3' similar to contains LTR2.t3 LTR2 repetitive element ;, mRNA sequence, internal tag
1186	CAGATAAGTT d	0	101	72	101	72	36131	collagen, type XIV, alpha 1 (undulin), reliable 3' end
1187	CATATCATTA d	0	94	21	94	21	119206	insulin-like growth factor binding protein 7, reliable 3' end
1188	TCACCGGTCA d	2	127	224	83	146	290070	gelsolin (amyloidosis, Finnish type), reliable 3' end
1189	AGGGAGCAGA d	0	77	76	77	76	296049	microfibrillar-associated protein, undefined 3' end
1190	CCCTTGTCCG d	0	75	60	75	60	127824	Homo sapiens cDNA FLJ36047 fis, clone TESTI2017951, reliable 3' end
1191	ATAAAAAGAA d	0	73	19	73	19	83942	cathepsin K (pycnodysostosis), reliable 3' end
1192	GTTGTCTTTG d	0	62	26	62	26	258798	Hypothetical protein FLJ20003, reliable 3' end
1193	CCGGGGGAGC d	0	61	110	61	110	172928	collagen, type I, alpha 1, internal tag
1194	TGGCCAGCTC d	2	92	64	60	42	AW572523	xw56a11.x2 NCI_CGAP_Pan1 Homo sapiens cDNA clone IMAGE:2831996 3', mRNA sequence, reliable 3' end
1195	TTCGGTTGGT d	0	59	19	59	19	BG939135	cn30g02.x1 Normal Human Trabecular Bone Cells Homo sapiens cDNA clone NHTBC_cn30g02 random, mRNA sequence, undefined 3' end
1196	TCAACTTCTG d	0	58	62	58	62	N57419	yw82e04.r1 Soares_placenta_8to9weeks_2NbHP8to9W Homo sapiens cDNA clone IMAGE:258750 5' similar to gb:M20681 GLUCOSE TRANSPORTER TYPE 3, BRAIN (HUMAN);contains Alu repetitive element;, mRNA sequence, undefined 3' end
1197	ACCCCCCCGC d	5	253	1029	55	223	2780	jun D proto-oncogene, undefined 3' end
1198	GTGCGCTGAG d	0	52	33	52	33	277477	HLA-C Major histocompatibility complex, class I, C, reliable 3' end.
1199	GACCAGCAGA d	0	48	43	48	43	172928	collagen, type I, alpha 1, internal tag
1200	GTCAAAATTT d	0	47	110	47	110	108623	thrombospondin 2, reliable 3' end
1201	GTGCTAAGCG d	3	141	308	46	100	159263	collagen, type VI, alpha 2, reliable 3' end
1202	ATTTCTTCAA d	0	44	19	44	19	AF311912	Homo sapiens pancreas tumor-related protein (FKSG12) mRNA, complete cds, undefined 3' end
1203	ACATTCTTTT d	0	44	17	44	17	82226	GPNMB Glycoprotein (transmembrane) nmb, reliable 3' end
1204	GGCACCTCAG d	2	65	36	42	23	93913	interleukin 6 (interferon, beta 2), reliable 3' end
1205	ACATTCCAAG d	0	42	50	42	50	245188	tissue inhibitor of metalloproteinase 3 (Sorsby fundus dystrophy, pseudoinflammatory), shorter alternative transcript
1206	AAAACGTTTT d	0	40	117	40	117	25647	FOS V-fos FBJ murine osteosarcoma viral oncogene homolog, internal tag
1207	TCCAGGAAAC d	0	39	72	39	72	11590	cathepsin F, reliable 3' end

Table 8. Genes differentially expressed in myoepithelial cells from DCIS and normal breast tissue

SEQ ID NO:	Tag_Sequence	NL	D6	D7	6/n	d7/n	Unigene	Gene
1208	CCTCCCAGCT d	2	58	74	38	48	98508	KIAA0150 protein, internal tag (NCBI only)
1209	CTTGGGTTTT d	0	37	122	37	122	251664	Homo sapiens cDNA FLJ22066 fis, clone HEP10611, reliable 3' end
1210	CCAGGGGAGA d	0	37	48	37	48	278613	interferon alpha-inducible protein 27, reliable 3' end
1211	GGGAGGGGTG d	3	113	100	37	33	R09745	yf27d09.s1 Soares fetal liver spleen 1NFLS Homo sapiens cDNA clone IMAGE:128081 3', mRNA, undefined 3' end
1212	GCACGGAAAA d	0	36	31	36	31	BG236552	nai45b05.x1 NCI_CGAP_HN20 Homo sapiens cDNA clone IMAGE:4263104 3', mRNA sequence, undefined 3' end
1213	GATGAGGAGA d	3	107	74	35	24	179573	retinoblastoma binding protein 1, internally primed site
1214	TGGAAAGTGA d	14	468	654	34	47	25647	FOS V-fos FBJ murine osteosarcoma viral oncogene homolog, reliable 3' end
1215	CGCCGACGAT d	0	32	100	32	100	265827	G1P3 interferon alpha-inducible protein, reliable 3' end
1216	CTGTCAGCGT d	0	32	29	32	29	283713	collagen triple helix repeat containing 1, reliable 3' end
1217	GTTCCACAGA d	0	32	24	32	24	179573	retinoblastoma binding protein 1, internally primed site
1218	GGAACTTTTA d	2	47	33	31	22	43857	similar to glucosamine-6-sulfatases, reliable 3' end
1219	GTATAAACGT d	0	31	29	31	29		No match
1220	GAGGAGGAGA d	0	30	26	30	26	78054	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 38, internal tag
1221	GGGGGGGGGT d	0	29	131	29	131	224731	EST, Weakly similar to 1203377A lamin A [Homo sapiens], reliable 3' end
1222	TTGGGATGGG d	0	29	103	29	103	278568	H factor (complement)-like 1, reliable 3' end
1223	TTCCGGTTCC d	0	29	17	29	17	172609	nucleobindin 1, reliable 3' end
1224	GGAAAGTGTT d	0	29	17	29	17	AW754264	PM4-CT0331-251199-001-F10 CT0331 Homo sapiens cDNA, mRNA sequence, undefined 3' end
1225	GCCCAGCTGG d	0	28	62	28	62	334798	hypothetical protein FLJ20897, reliable 3' end
1226	TTTCCCTCAA d	2	42	21	27	14	75111	protease, serine, 11 (IGF binding), reliable 3' end
1227	GGATGTGAAA d	0	26	19	26	19	177543	MIC2 antigen identified by monoclonal antibodies 12E7, F21 and O13, reliable 3' end
1228	GCAAAAAAAA d	5	120	143	26	31	4746	Hypothetical protein FLJ21324 reliable 3' end
1229	ACCCACGTCA d	5	113	317	25	69	198951	jun B proto-oncogene, reliable 3' end
1230	CGGGGTGGCC d	0	24	193	24	193	1584	cartilage oligomeric matrix protein (pseudoachondroplasia, epiphyseal dysplasia 1, multiple), reliable 3' end
1231	CGCCCCGGCG d	0	24	43	24	43	BM145074	TCAAP1D14680 Pediatric acute myelogenous leukemia cell (FAB M1) Baylor-HGSC project=TCAA Homo sapiens cDNA clone TCAAP1468, mRNA sequence, reliable 3' end
1232	CAGACTTTTG d	0	24	24	24	24	63348	elastin microfibril interface located protein, reliable 3' end
1233	TTACTTCTGC d	0	23	45	23	45	75736	apolipoprotein D, internal tag
1234	CGTCTTTAAA d	0	23	26	23	26	21275	Hypothetical protein FLJ11011, internal tag
1235	TTGCTGACTT d	12	279	122	23	10	108885	collagen, type VI, alpha 1, reliable 3'end
1236	TCGAAGAACC d	2	34	60	22	39	76294	CD63 antigen (melanoma 1 antigen) reliable 3'end
1237	GGCCCCTCAC d	0	22	74	22	74	274313	insulin-like growth factor binding protein 6, reliable 3' end
1238	CAGCTGGCCA d	0	22	36	22	36	79732	fubulin, transcript variant C, reliable 3' end
1239	TGTAAACAAT d	0	22	19	22	19	170040	platelet-derived growth factor receptor-like, reliable 3' end

Table 8. Genes differentially expressed in myoepithelial cells from DCIS and normal breast tissue

SEQ ID NO:	Tag_Sequence	NL	D6	D7	6/n	d7/n	Unigene	Gene
1240	GAGATCCGCA d	0	21	62	21	62	75348	proteasome (prosome, macropain) activator subunit 1 (PA28 alpha), reliable 3' end
1241	CCCTGGGTTC d	6	124	74	20	12	111334	FTL Ferritin, light polypeptide, reliabe 3' end
1242	CTAACGGGGC d	0	20	169	20	169	102171	immunoglobulin superfamily containing leucine-rich repeat, reliable 3' end
1243	TGCGCTCTCC d	0	20	86	20	86	25391	Homo sapiens, clone IMAGE:4691115, mRNA, partial cds, reliable 3' end
1244	CGCAGTCTGC d	0	20	48	20	48	24087	Arylhydrocarbon receptor repressor, internal tag
1245	GGAGGAATTC d	0	20	21	20	21	78056	cathepsin L, reliable 3' end
1246	AAGAAAGGAG d	0	20	21	20	21	202097	procollagen C-endopeptidase enhancer, reliable 3' end
1247	ACTTATTATG d	2	30	107	19	70	76152	decorin, reliable 3' end
1248	TAGTTGGAAA d	9	173	105	19	11	1119	nuclear receptor subfamily 4, group A, member 1, reliable 3' end
1249	TCAACAAATT d	0	19	48	19	48	9315	HNOEL-iso protein, reliable 3' end
1250	GCGTGAGTGC d	0	19	17	19	17	AW894414	CM2-NN0032-050400-142-g12 NN0032 Homo sapiens cDNA, mRNA sequence, undefined 3' end
1251	CGGCTGAATT d	0	19	17	19	17	75888	phosphogluconate dehydrogenase, reliable 3' end
1252	AGCAAACTGA d	0	19	17	19	17	182579	leucine aminopeptidase 3, reliable 3' end
1253	GCGCAGAGGT d	15	277	148	18	10	BQ344433	MR2-NT0136-161100-003-a05 NT0136 Homo sapiens cDNA, mRNA sequence, undefined 3' end
1254	TGGGACTCCA d	2	28	45	18	30	59384	hypothetical protein MGC3047, reliable 3' end
1255	ACTCAGCCCG d	2	28	36	18	23	101382	tumor necrosis factor, alpha-induced protein 2, reliable 3' end
1256	CAGCACGGAT d	2	28	26	18	17		No match
1257	GGAAATGTCA d	18	325	93	18	5	111301	Matrix metalloproteinase 2 (gelatinase A, 72kD gelatinase, 72kD type IV collagenase, reliable 3' end
1258	TGCGCTGGCC d	0	18	67	18	67	289019	latent transforming growth factor beta binding protein 3, reliable 3' end
1259	GACGGCTGCA d	2	26	74	17	48	258730	Heme-regulated initiation factor 2-alpha kinase, undefined 3' end
1260	GGAAGTTTCG d	2	26	36	17	23	55847	mitochondrial ribosomal protein L51, reliable 3' end
1261	GGGCCAACCC d	0	17	88	17	88	119475	Cold inducible RNA binding protein, undefined 3' end
1262	GACGCGGCGC d	0	17	24	17	24	352987	MGC21945 Binder of Rho GTPase 3-like, reliable 3' end
1263	TATCCTGAAA d	0	17	17	17	17	AA778363	zl56g03.s1 Soares_pregnant_uterus_NbHPU Homo sapiens cDNA clone IMAGE:505972 3' similar to contains L1.t3 L1 repetitive element ;, mRNA sequence, undefined 3' end
1264	ATGGCAACAG d	0	17	17	17	17	149609	integrin, alpha 5 (fibronectin receptor, alpha polypeptide), reliable 3'end
1265	ACGACAAAGC d	0	17	17	17	17	83920	peptidylglycine alpha-amidating monooxygenase, reliable 3' end
1266	ACTGAAAGAA d	3	50	124	16	40	169756	C1S Complement component 1, s subcomponent, reliable 3' end
1267	GGCTGCCCTG d	2	24	62	16	40	74566	Dihydropyrimidinase-like 3, reliable 3' end
1268	GGCACGCAGC d	0	15	79	15	79	BF349813	RC1-HT0217-151099-011-e05 HT0217 Homo sapiens cDNA, mRNA sequence, undefined 3' end
1269	CAAAAAATTA d	0	15	43	15	43	H81706	ys67c09.r1 Soares retina N2b4HR Homo sapiens cDNA clone IMAGE:219856 5', mRNA sequence, undefined 3' end
1270	GGCCACGTAG d	0	15	26	15	26	155597	DF D component of complement (adipsin), internal tag
1271	CTAAAAAAAA d	0	15	26	15	26	54457	CD81 antigen (target of antiproliferative antibody 1), reliable 3' end

Table 8. Genes differentially expressed in myoepithelial cells from DCIS and normal breast tissue

SEQ ID NO:	Tag_Sequence	NL	D6	D7	6/n	d7/n	Unigene	Gene
1272	CCAAGGTTTT d	0	15	19	15	19	99120	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide, Y chromosome, internal tag
1273	GACAAAAAAA d	6	91	33	15	5	32366	DERMO1 Likely ortholog of mouse and rat twist-related bHLH protein Dermo-1, reliable 3' end
1274	CCCTACCCTG d	11	160	792	15	74	75736	apolipoprotein D, reliable 3' end
1275	GGAAAAAAAA d	3	45	93	15	30	198271	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 10 (42kD), reliable 3' end
1276	GCGGCGGCTC d	2	22	26	14	17	BQ339816	RC5-NN1165-251100-024-F08 NN1165 Homo sapiens cDNA, mRNA sequence, undefined 3' end
1277	GCGAAACCCA d	0	14	67	14	67	359286	ESTs, Moderately similar to hypothetical protein FLJ20378 [Homo sapiens], reliable 3' end
1278	CTAATAAACT d	0	14	17	14	17	279583	CGI-81 protein, shorter alternative transcript
1279	AAGAGCGCCG d	12	172	45	14	4	8997	Sad1 unc-84 domain protein 1, reliable 3' end
1280	GCTGAACGCG d	14	193	60	14	4	99029	CCAAT/enhancer binding protein (C/EBP), beta, reliable 3' end
1281	GCCCCCAATA d	29	400	270	14	9	227751	lectin, galactoside-binding, soluble, 1 (galectin 1), reliable 3' end
1282	GCGGGGTGGA d	6	83	177	13	29	85155	zinc finger protein 36, C3H type-like 1, internally primed site
1283	TAGTTGGAAC d	5	62	41	13	9	BG057763	7f75e10.x1 Lupski_dorsal_root_ganglion Homo sapiens cDNA clone IMAGE:3302875 3', mRNA, reliable 3' end
1284	CAAGTTCTTT d	3	41	60	13	19	356629	Homo sapiens cDNA FLJ31414 fis, clone NT2NE2000260, weakly similar to THYMOSIN BETA-4, undefined 3' end
1285	CGACCCCACG d	6	81	60	13	10	169401	apolipoprotein E, undefined 3' end
1286	GAATTCACAA d	0	13	131	13	131	128087	F2R coagulation factor II (thrombin) receptor, reliable 3' end
1287	GAGTGGGTGC d	0	13	69	13	69	12908	CDC42 binding protein kinase beta (DMPK-like), undefined 3' end
1288	CAGCGGCGGG d	0	13	57	13	57	2420	superoxide dismutase 3, extracellular, reliable 3' end
1289	GCCTGTCCCT d	0	13	50	13	50	821	biglycan, reliable 3' end
1290	CAGGACAGTT d	0	13	48	13	48	78305	RAB2, member RAS oncogene family, shorter alternative transcript
1291	GCAGAAAATT d	0	13	21	13	21	333555	echinoderm microtubule associated protein like 4, reliable 3' end
1292	CATAAATGCG d	0	13	21	13	21	237356	stromal cell-derived factor 1, SAGE Genie: no match, NCBI: Acc.no.U19495
1293	GTGGCAGCGC d	0	13	17	13	17	285753	stathmin-like 3, reliable 3' end
1294	CACACAGTTT d	6	80	98	13	16	204354	ras homolog gene family, member B, undefined 3' end
1295	GGTGCCCAGT d	2	20	76	13	50	75607	myristoylated alanine-rich protein kinase C substrate, internally primed site
1296	TTCTGTGCTG d	3	40	105	13	34	1279	C1R Complement component 1, r subcomponent, reliable 3' end
1297	CTCTCCAAAC d	2	20	26	13	17	151242	serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1, (angioedema, hereditary), reliable 3' end
1298	GGCCCTAGGC d	3	39	98	13	32	78909	zinc finger protein 36, C3H type-like 2, reliable 3' end
1299	CTCAACCCCC d	2	19	105	12	68	89137	Low density lipoprotein-related protein 1 (alpha-2-macroglobulin receptor), reliable 3' end
1300	AGCCACCGCG d	2	19	43	12	28	193716	Complement component (3b/4b) receptor 1, including Knops blood group system, reliable 3' end
1301	ACCTTGAAGT d	2	19	36	12	23	29352	tumor necrosis factor, alpha-induced protein 6, internally primed site

Table 8. Genes differentially expressed in myoepithelial cells from DCIS and normal breast tissue

SEQ ID NO:	Tag_Sequence	NL	D6	D7	6/n	d7/n	Unigene	Gene
1302	TCAGAAGTTT d	2	19	29	12	19	243901	Homo sapiens mRNA; cDNA DKFZp564C1563 (from clone DKFZp564C1563), reliable 3' end
1303	TGGCAAAATA d	2	19	26	12	17	BM353720	ig55c02.y1 HR85 islet Homo sapiens cDNA 5', mRNA sequence, undefined 3' end
1304	GGGAGGTAGC d	2	18	31	11	20	171825	Basic helix-loop-helix domain containing, class B, 2, reliable 3' end
1305	GAAAAATTTA d	5	50	86	11	19	169248	cytochrome c, reliable 3' end
1306	GGCAGGCGGG d	6	65	55	11	9	333069	Ets2 repressor factor, reliable 3' end
1307	AGATTCAAAC d	3	32	41	10	13	14368	SH3 domain binding glutamic acid-rich protein like, reliable 3' end
1308	GTAAAAAAAA d	8	78	86	10	11	460	Activating transcription factor 3, reliable 3'end (+at least 10 others)
1309	AGGCTCCTGG d	3	31	217	10	71	24395	small inducible cytokine subfamily B (Cys-X-Cys), member 14 (BRAK), reliable 3' end
1310	CGCCGCGGTG d	3	31	48	10	16	4835	eukaryotic translation initiation factor 3, subunit 8 (110kD), reliable 3' end
1311	TGCCTGCACC d	5	46	76	10	17	135084	cystatin C (amyloid angiopathy and cerebral hemorrhage), reliable 3' end
1312	GTGACTGCCA d	5	45	38	10	8	84183	Diptheria toxin resistance protein required for diphthamide biosynthesis-like 1 (S. cerevisiae), reliable 3' end
1313	GTTTATGGAT d	3	30	26	10	9	365706	matrix Gla protein, reliable 3' end
1314	GCAGCCATCC d	34	321	334	10	10	4437	ribosomal protein L28, reliable 3' end
1315	CAGGTTTCAT d	12	117	124	10	10	24395	small inducible cytokine subfamily B (Cys-X-Cys), member 14 (BRAK), reliable 3' end
1316	GGCCTGCTGC d	6	58	45	10	7	9634	Hypothetical protein BC009925, reliable 3' end
1317	CCCCCTGGAT d	6	56	119	9	19	275243	S100 calcium binding protein A6 (calcyclin), reliable 3' end
1318	GGGGGAATTT d	3	28	124	9	40	BM805435	AGENCOURT_6498312 NIH_MGC_124 Homo sapiens cDNA clone IMAGE:5728837 5', mRNA, undefined 3' end
1319	AACTTTTGGC d	3	28	55	9	18	195471	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3, internally primed site
1320	AGAATTTGCA	6	53	50	9	8	250655	prothymosin, alpha (gene sequence 28), internally primed site
1321	GCCGCCCTGC	5	40	33	9	7	82208	ACADVL Acyl-Coenzyme A dehydrogenase, very long chain, reliable 3'end
1322	GGGGGTAACT	5	39	38	8	8	99969	fusion, derived from t(12;16) malignant liposarcoma, reliable 3' end
1323	TGAAAAAAAA	5	35	33	8	7	119178	Cation-chloride cotransporter-interacting protein, reliable 3' end
1324	GGCCTTTTTT	5	35	29	8	6	109804	H1FX H1 histone family, member X, reliable 3' end
1325	GCGACGAGGC	14	95	91	7	7	2017	ribosomal protein L38, internal tag
1326	GCGCTGGAGT d	3	21	33	7	11	110695	hypothetical protein MGC3133, reliable 3' end
1327	GGAGGGGGCT	9	62	48	7	5	77886	Lamin A/C, internally primed site
1328	GAGGGAGTTT	152	993	964	7	6	76064	ribosomal protein L27a, reliable 3' end
1329	CGCTGGTTCC	37	237	184	6	5	179943	ribosomal protein L11, reliable 3' end
1330	TCAAGCCATC	9	58	45	6	5	BG060046	naf48a07.x1 NCI_CGAP_Brn65 Homo sapiens cDNA clone IMAGE:4147116 3', mRNA sequence, undefined 3' end
1331	GGCTTTGGAG d	5	29	64	6	14	90918	C11orf10 Chromosome 11 open reading frame 10, reliable 3' end
1332	CTGCCAAGTT	14	85	81	6	6	75873	Zyxin, reliabe 3' end
1333	GACTCACTTT	11	65	50	6	5	699	peptidylprolyl isomerase B (cyclophilin B), reliable 3' end
1334	GGGGAAATCG d	34	195	544	6	16	76293	thymosin, beta 10, internally primed site

Table 8. Genes differentially expressed in myoepithelial cells from DCIS and normal breast tissue

SEQ ID NO:	Tag_Sequence	NL	D6	D7	6/n	d7/n	Unigene	Gene
1335	GGCCGCGTTC d	20	115	568	6	28	5174	ribosomal protein S17, reliable 3' end
1336	CCGTGACTCT	12	70	112	6	9	296267	follistatin-like 1, reliable 3' end
1337	TGCACGTTTT	117	631	453	5	4	169793	ribosomal protein L32, reliable 3' end
1338	GTTGTGGTTA	81	429	274	5	3	75415	beta-2-microglobulin, reliable 3' end
1339	GTTAACGTCC	11	54	100	5	9	178391	ribosomal protein L36a, reliable 3' end
1340	CAGGAGTTCA	6	30	50	5	8	83583	Actin related protein 2/3 complex, subunit 2 (34 kD), reliable 3' end
1341	CCTCGGAAAA d	15	74	224	5	15	2017	ribosomal protein L38, reliable 3' end
1342	CCCGTCCGGA d	81	388	1002	5	12	180842	ribosomal protein L13, reliable 3' end
1343	GGAAGCTAAG	34	150	181	4	5	136348	Osteoblast specific factor 2 (fasciclin I-like), undefined 3' end
1344	CCCATCCGAA	29	129	179	4	6	91379	ribosomal protein L26, reliable 3' end
1345	CCCCAGCCAG	18	77	98	4	5	252259	Ribosomal protein S3, reliable 3' end
1346	GGTGGCACTC	11	43	81	4	8	77273	ras homolog gene family, member A, reliable 3' end
1347	ATGGTGGGGG	51	200	172	4	3	343586	zinc finger protein 36, C3H type, homolog (mouse), reliable 3' end
1348	CGCCGCCGGC	68	265	442	4	7	182825	ribosomal protein L35, reliable 3' end
1349	CAGCAGAAGC	9	35	45	4	5	26703	CCR4-NOT transcription complex, subunit 8, reliable 3' end
1350	TTGGGGTTTC	158	555	515	4	3	62954	Ferritin, heavy polypeptide 1, reliable 3' end
1351	CCAGTGGCCC d	14	47	134	3	10	180920	ribosomal protein S9, reliable 3' end
1352	CGCCGGAACA	29	95	148	3	5	286	ribosomal protein L4, reliable 3' end
1353	CTGTACTTGT	18	56	98	3	5	75678	FBJ murine osteosarcoma viral oncogene homolog B, reliable 3' end
1354	ACCATCCTGC	25	68	76	3	3	76095	immediate early response 3, reliable 3' end
1355	GTGAAACTCC	21	58	93	3	4	BI005171	PM3-HN0076-020401-008-d01 HN0076 Homo sapiens cDNA, mRNA sequence, reliable 3' end
1356	GCCGTGTCCG	63	151	379	2	6	350166	ribosomal protein S6, reliable 3' end
1357	GCGAAACCCC	48	113	198	2	4	30211	hypothetical protein FLJ22313, reliable 3' end
1358	GCCGAGGAAG	55	111	260	2	5	339696	ribosomal protein S12, reliable 3' end
1359	TTGAATTCCC d	44	15	2	-3	-19	171921	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C, reliable 3' end
1360	GTGCTGAATG	144	50	29	-3	-5	77385	myosin, light polypeptide 6, alkali, smooth muscle and non-muscle, reliable 3' end
1361	TTGAAGCTTT d	451	154	19	-3	-24	75765	GRO2 oncogene, reliable 3' end
1362	GCATAATAGG d	270	89	14	-3	-19	350077	ribosomal protein L21, reliable 3' end
1363	AAGACAGTGG	137	44	26	-3	-5	296290	ribosomal protein L37a, reliable 3' end
1364	TGTTCTGGAG	75	24	19	-3	-4	74471	Gap junction protein, alpha 1, 43kD (connexin 43), reliable 3' end
1365	ACAGGCTACG	100	31	38	-3	-3	75777	transgelin, reliable 3' end
1366	AAGAAGATAG	77	23	12	-3	-6	182426	Ribosomal protein S2, reliable 3' end
1367	GACTTGTATA	44	13	5	-3	-9	81328	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha, internally primed site
1368	ATTCTCCAGT	121	35	17	-3	-7	234518	ribosomal protein L23, reliable 3' end
1369	TTATGGGGAG d	32	9	0	-4	-32	75612	stress-induced-phosphoprotein 1 (Hsp70/Hsp90-organizing protein), reliable 3' end

Table 8. Genes differentially expressed in myoepithelial cells from DCIS and normal breast tissue

SEQ ID NO:	Tag_Sequence	NL	D6	D7	6/n	d7/n	Unigene	Gene
1370	GGCTGTACCC	118	32	26	-4	-4	BC007492	Homo sapiens, cysteine and glycine-rich protein 1, clone IMAGE:2966961, mRNA, reliable 3' end
1371	ATGGCTGGTA	156	42	19	-4	-8	182426	ribosomal protein S2, reliable 3' end
1372	TGAAGTTATA	71	19	24	-4	-3	287797	integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12), reliable 3' end
1373	AGTATGAGGA	64	17	7	-4	-9	211600	Tumor necrosis factor, alpha-induced protein 3, reliable 3' end
1374	GCCTACCCGA	74	19	12	-4	-6	23582	tumor-associated calcium signal transducer 2, reliable 3' end
1375	CGTGTTAATG d	26	7	2	-4	-11	2110	zinc finger protein 9 (a cellular retroviral nucleic acid binding protein), reliable 3' end
1376	TTGTAATCGT d	57	14	2	-4	-24	NM_004152	Homo sapiens ornithine decarboxylase antizyme 1 (OAZ1), mRNA, reliable 3' end
1377	TCTTGTGCAT	32	8	5	-4	-7	2795	lactate dehydrogenase A, reliable 3' end
1378	TTACCATATC d	74	18	7	-4	-10	300141	ribosomal protein L39, reliable 3' end
1379	TGGAAGCACT d	94	22	7	-4	-13	624	interleukin 8, reliable 3' end
1380	CTGCTATACG	91	21	21	-4	-4	180946	Ribosomal protein L5, reliable 3' end
1381	TGCTGTGCAT d	72	17	0	-4	-72	75692	Asparagine synthetase, reliable 3' end
1382	ACTAACACCC	63	14	14	-4	-4	BC009321	Homo sapiens, clone MGC:16650 IMAGE:4123521, mRNA, complete cds, reliable 3' end
1383	GATCTCTTGG d	29	7	0	-4	-29	38991	S100 calcium binding protein A2, reliable 3' end
1384	TACTCTTGGC d	25	6	0	-4	-25	2730	heterogeneous nuclear ribonucleoprotein L, reliable 3' end
1385	CTGTTGATTG	51	11	10	-5	-5	249495	heterogeneous nuclear ribonucleoprotein A1, shorter alternative transcript
1386	TAATAAAGGT d	180	39	7	-5	-25	151604	ribosomal protein S8, reliable 3' end
1387	CCACTGCACT	321	67	67	-5	-5	68257	General transcription factor IIF, polypeptide 1 (74kD subunit), reliable 3' end
1388	AGAAAGATGT d	229	47	10	-5	-24	78225	annexin A1, reliable 3' end
1389	CTGTACAGAC d	43	9	5	-5	-9	251653	tubulin, beta, 2, reliable 3' end
1390	AGAAATGTTG d	28	6	0	-5	-28	146217	Homo sapiens cDNA FLJ34184 fis, clone FCBBF3017024, reliable 3' end
1391	GGCTTTACCC d	74	14	0	-5	-74	119140	eukaryotic translation initiation factor 5A, reliable 3' end
1392	ACAGTGGGGA d	57	11	2	-5	-24	278270	unactive progesterone receptor, 23 kD, reliable 3' end
1393	TGTATAAAAA d	40	8	2	-5	-17	82689	tumor rejection antigen (gp96) 1, reliable 3' end
1394	TTATGGGATC	63	12	19	-5	-3	5662	guanine nucleotide binding protein (G protein), beta polypeptide 2-like 1, reliable 3' end
1395	TTACTAAATG d	23	4	0	-5	-23	155560	Calnexin, reliable 3' end
1396	GCCTTGGGTG d	81	15	0	-5	-81	2250	leukemia inhibitory factor (cholinergic differentiation factor), reliable 3' end
1397	ATCAAGGGTG	92	17	14	-6	-6	157850	ribosomal protein L9, reliable 3' end
1398	TAGGTAGCTC d	25	4	0	-6	-25	179999	Homo sapiens, clone IMAGE:3457003, mRNA, reliable 3' end
1399	TACCATCAAT d	198	35	14	-6	-14	169476	glyceraldehyde-3-phosphate dehydrogenase, reliable 3' end
1400	CATTTGTAAT	32	6	5	-6	-7	X93334	mitochondrial
1401	AAACTGTGGT d	20	3	0	-6	-20	W31349	zb95d06.s1 Soares_parathyroid_tumor_NbHPA Homo sapiens cDNA clone IMAGE:320555 3' similar to S W:COX2_GORGO P26456 CYTOCHROME C OXIDASE POLYPEPTIDE II ;, mRNA sequence, undefined 3'end

Table 8. Genes differentially expressed in myoepithelial cells from DCIS and normal breast tissue

SEQ ID NO:	Tag_Sequence	NL	D6	D7	6/n	d7/n	Unigene	Gene
1402	AAGCTGTATA d	34	6	0	-6	-34	289114	hexabrachion (tenascin C, cytotactin), reliable 3' end
1403	TAAAACAAGA d	41	7	2	-6	-17	1369	Decay accelerating factor for complement (CD55, Cromer blood group system), reliable 3' end
1404	TGATATGTCA d	49	8	0	-6	-49	AI969049	wq70c08.x1 NCI_CGAP_GC6 Homo sapiens cDNA clone IMAGE:2476622 3' similar to gb:M36820 MACROPHAGE INFLAMMATORY PROTEIN-2-ALPHA PRECURSOR (HUMAN);; mRNA sequence, undefined 3' end
1405	CGAATGTCCT d	72	11	0	-7	-72	335952	keratin 6B, reliable 3' end
1406	GTGCGCCGGA d	61	9	0	-7	-61	BQ378038	QV0-UM0093-250800-360-c02 UM0093 Homo sapiens cDNA, mRNA sequence, undefined 3' end
1407	GCAACTTAGA d	80	11	7	-7	-11	54451	Laminin, gamma 2 (nicein (100kD), kalinin (105kD), BM600 (100kD), shorter alternative transcript
1408	TCTCTACTAA d	49	7	5	-7	-10	250641	Tropomyosin 4, reliable 3' end
1409	CCTCAGGATA d	25	3	0	-7	-25	BC012090	Homo sapiens, Similar to heterogeneous nuclear ribonucleoprotein A3, clone MGC:20045 IMAGE:4661041, mRNA, complete cds, reliable 3' end
1410	TCTGTAATCC d	34	4	0	-8	-34	142	sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1, reliable 3' end
1411	TCCTGTAAAG d	34	4	0	-8	-34	74034	Caveolin 1, caveolae protein, 22kD, reliable 3' end
1412	GTGTAATAAG d	77	10	2	-8	-32	232400	Heterogeneous nuclear ribonucleoprotein A2/B1, reliable 3' end
1413	TAGCTCTATG d	43	6	0	-8	-43	76549	ATPase, Na+/K+ transporting, alpha 1 polypeptide, reliable 3' end
1414	CTTTCTTTGA d	35	4	2	-8	-15	4909	Dickkopf homolog 3 (Xenopus laevis), reliable 3' end
1415	CTTGAGCAAT d	63	8	0	-8	-63	848	FK506 binding protein 4 (59kD), reliable 3' end
1416	AGGCCTCGGC d	28	3	2	-8	-12	301885	Homo sapiens cDNA FLJ33794 fis, clone CTONG1000009, undefined 3' end
1417	TTCTTGTTTT d	57	7	5	-9	-12	74621	Prion protein (p27-30) (Creutzfeld-Jakob disease, Gerstmann-Strausler-Scheinker syndrome, fatal familial insomnia) reliable 3' end
1418	TGTAGGTCAT d	29	3	0	-9	-29	111554	ADP-ribosylation factor-like 7, reliable 3' end
1419	TTAAGACTTC d	49	6	0	-9	-49	136309	SH3-domain GRB2-like endophilin B1, internal tag
1420	GGGTTGGCTT d	118	13	19	-9	-6	348493	LOC114928 Hypothetical protein BC013576, internal tag
1421	GTACTAGTGT d	89	10	5	-9	-19	303649	small inducible cytokine A2 (monocyte chemotactic protein 1), reliable 3' end
1422	GTTTTTGCTT d	20	2	0	-9	-20	7718	hypothetical protein FLJ22678, reliable 3' end
1423	GGGGCACTTG d	20	2	0	-9	-20	54451	Laminin, gamma 2 (nicein (100kD), kalinin (105kD), BM600 (100kD), Herlitz junctional epidermolysis bullosa)), reliable 3' end
1424	CTCAGTCTTT d	20	2	0	-9	-20	AW304910	xv90h12.x1 NCI_CGAP_Brn53 Homo sapiens cDNA clone IMAGE:2825831 3', mRNA sequence, undefined 3' end
1425	AATATTGAGA d	31	3	2	-9	-13	106673	eukaryotic translation initiation factor 3, subunit 6 (48kD), reliable 3' end
1426	TTATAAAAGA d	21	2	0	-10	-21	BG009283	RC4-GN0321-011200-011-c02 GN0321 Homo sapiens cDNA, mRNA sequence, undefined 3' end
1427	TATAAGGTGG d	21	2	0	-10	-21	169531	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 21, reliable 3' end
1428	TACTGGAAGT d	21	2	0	-10	-21	9075	serine/threonine kinase 17a (apoptosis-inducing), internally primed site
1429	CTTTCAGATG d	21	2	0	-10	-21	99910	phosphofructokinase, platelet, reliable 3' end
1430	TCACTGCACT d	68	7	0	-10	-68	287617	Homo sapiens cDNA FLJ14058 fis, clone HEMBB1000554, undefined 3' end

Table 8. Genes differentially expressed in myoepithelial cells from DCIS and normal breast tissue

SEQ ID NO:	Tag_Sequence	NL	D6	D7	6/n	d7/n	Unigene	Gene
1431	TTAATATATG d	23	2	0	-10	-23	356386	RAB7, member RAS oncogene family, reliable 3' end
1432	TTCATACACC d	350	33	19	-11	-18	X93334	mitochondrial
1433	TACTAGTCCT d	48	4	0	-11	-48	BE969428	601649644R2 NIH_MGC_74 Homo sapiens cDNA clone IMAGE:3933371 3', mRNA sequence
1434	TGGATCAACC d	25	2	0	-11	-25	74034	caveolin 1, caveolae protein, 22kD, reliable 3' end
1435	TCCCTATTAA d	492	43	181	-11	-3		No match
1436	TACAAACGGT d	26	2	2	-12	-11	BG563838	602584639F1 NIH_MGC_76 Homo sapiens cDNA clone IMAGE:4712624 5', mRNA sequence, undefined 3' end
1437	TCAAATGCAT d	54	4	5	-12	-11	182447	Heterogeneous nuclear ribonucleoprotein C (C1/C2), reliable 3' end
1438	AGGTCTTCAA d	86	7	17	-13	-5	87409	thrombospondin 1, reliable 3' end
1439	CCTGGTCCCA d	43	3	5	-13	-9	23881	keratin 7, reliable 3' end
1440	TTTCCTCTCA d	130	10	0	-13	-130	184510	stratifin, reliable 3' end
1441	CTGTTGGCAT d	31	2	2	-14	-13	350077	Ribosomal protein L21, internally primed site
1442	TTTGTAGATG d	31	2	0	-14	-31	3069	heat shock 70kD protein 9B (mortalin-2), reliable 3' end
1443	TCATCATCTG d	32	2	2	-15	-13	116159	ESTs, reliable 3' end
1444	CCATTGCACT d	86	6	0	-16	-86	211563	B-cell CLL/lymphoma 7A, reliable 3' end
1445	GTCCTTTCTG d	54	3	0	-16	-54	799	diphtheria toxin receptor (heparin-binding epidermal growth factor-like growth factor), reliable 3' end
1446	CTTCCTTGCC d	1204	69	17	-17	-72	2785	keratin 17, reliable 3' end
1447	GTTTCATCTC d	38	2	0	-17	-38	1940	crystallin, alpha B, reliable 3' end
1448	AGTGTCTGTG d	135	8	29	-18	-5	8867	cysteine-rich, angiogenic inducer, 61, reliable 3' end
1449	ACCAGTGGTT d	20	1	0	-18	-20	AI857657	wk96a06.x1 NCI_CGAP_Lu19 Homo sapiens cDNA clone IMAGE:2423218 3' similar to gb:M93010 14-3-3 PROTEIN HOMOLOG STRATIFIN (HUMAN);contains element MSR1 MER22 repetitive element ;, mRNA sequence, undefined 3' end
1450	ACACTTGGAG d	40	2	0	-18	-40	BF980200	602288029T1 NIH_MGC_97 Homo sapiens cDNA clone IMAGE:4373839 3', mRNA sequence, internal tag
1451	GCTTAGAAGT d	41	2	0	-19	-41	289088	heat shock 90kD protein 1, alpha, internally primed site
1452	CAGAAGGCCA d	21	1	0	-20	-21	75668	Homo sapiens, Similar to RIKEN cDNA 1700018O18 gene, clone IMAGE:4121436, mRNA, partial cds, reliable 3' end
1453	TTTACTTTGG d	20	0	0	-20	-20	77889	Friedreich ataxia region gene X123, reliable 3' end
1454	TATCCCAACT d	20	0	0	-20	-20	AA729014	nw25h05.s1 NCI_CGAP_GCB0 Homo sapiens cDNA clone IMAGE:1241529 3', mRNA sequence, reliable 3' end
1455	CTGACTTGTG d	20	0	0	-20	-20	BF869689	IL3-ET0116-231000-299-H09 ET0116 Homo sapiens cDNA, mRNA sequence, undefined 3' end
1456	ACCTTTACTG d	20	0	0	-20	-20	77356	transferrin receptor (p90, CD71), reliable 3' end
1457	AAATACCTAA d	20	0	0	-20	-20	AW835549	QV4-LT0016-271299-068-h02 LT0016 Homo sapiens cDNA, mRNA sequence, undefined 3' end
1458	CTTAAGGATT d	46	2	2	-21	-19	165998	PAI-1 mRNA-binding protein, reliable 3' end

Table 8. Genes differentially expressed in myoepithelial cells from DCIS and normal breast tissue

SEQ ID NO:	Tag_Sequence	NL	D6	D7	6/n	d7/n	Unigene	Gene
1459	TTGGGTTAAT d	23	1	0	-21	-23	AW834375	MR2-TT0013-241199-018-d09 TT0013 Homo sapiens cDNA, mRNA sequence, undefined 3' end
1460	TATTTTTGTT d	23	1	0	-21	-23	9238	FLJ23516 Hypothetical protein FLJ23516, reliable 3' end
1461	GTGGATGGAC d	23	1	0	-21	-23	6418	seven transmembrane domain orphan receptor, reliable 3' end
1462	ATAGACATAA d	23	1	0	-21	-23	78614	complement component 1, q subcomponent binding protein, reliable 3' end
1463	AAGGCTGGAA d	23	1	0	-21	-23	85962	hyaluronan synthase 3, reliable 3' end
1464	TTTGTACACA d	21	0	0	-21	-21	BE963003	601656371R1 NIH_MGC_66 Homo sapiens cDNA clone IMAGE:3856313 3', mRNA sequence
1465	TGGGAAGAGG d	21	0	0	-21	-21	BG569626	602587323F1 NIH_MGC_76 Homo sapiens cDNA clone IMAGE:4716100 5', mRNA sequence, undefined 3' end
1466	GTATTTAACA d	21	0	0	-21	-21	9006	VAMP (vesicle-associated membrane protein)-associated protein A (33kD), reliable 3' end
1467	GGAAAGATGT d	21	0	0	-21	-21	9398	FLJ10055 Hypothetical protein FLJ10055, internal tag
1468	TGGAGAATGT d	23	0	0	-23	-23	287797	ITGB1 Integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12), internally primed site
1469	TATGTATGTT d	23	0	0	-23	-23	283738	casein kinase 1, alpha 1, reliable 3' end
1470	TACCTAATTG d	23	0	0	-23	-23	BF896098	CM2-MT0158-221100-551-c04 MT0158 Homo sapiens cDNA, mRNA sequence, undefined 3' end
1471	TAATAAAGCA d	23	0	0	-23	-23	4888	seryl-tRNA synthetase, reliable 3' end
1472	GTACTGTATG d	23	0	0	-23	-23	180446	karyopherin (importin) beta 1, reliable 3' end
1473	GCTGTAGCCA d	23	0	0	-23	-23	BM145758	TCAAP1D7727 Pediatric acute myelogenous leukemia cell (FAB M1) Baylor-HGSC project=TCAA Homo sapiens cDNA clone TCAAP7727, mRNA sequence, reliable 3' end
1474	TTAGATAAGC d	26	1	0	-24	-26	82916	chaperonin containing TCP1, subunit 6A (zeta 1), reliable 3' end
1475	TCATAATAGG d	25	0	0	-25	-25		No match
1476	TAATTTATAG d	25	0	0	-25	-25		No match
1477	GGTCACTGAG d	25	0	0	-25	-25	254105	enolase 1, (alpha), internal tag
1478	CCTTTTTCAA d	25	0	0	-25	-25	AI687998	wa77h02.x1 Soares_NFL_T_GBC_S1 Homo sapiens cDNA clone IMAGE:2302227 3' similar to S W:COX1_HUMAN P00395 CYTOCHROME C OXIDASE POLYPEPTIDE I ;, mRNA sequence, undefined 3' end
1479	ACTACTAAGG d	25	0	0	-25	-25	2820	oxytocin receptor, reliable 3' end
1480	GATGTGCACG d	520	21	12	-25	-44	117729	keratin 14 (epidermolysis bullosa simplex, Dowling-Meara, Koebner), reliable 3' end
1481	TTCTTTTCAT d	26	0	0	-26	-26	4310	eukaryotic translation initiation factor 1A, reliable 3' end
1482	CGAAAGATGT d	26	0	0	-26	-26		No match
1483	AAAGTCATTG d	60	2	0	-27	-60	77899	tropomyosin 1 (alpha), internal tag
1484	TGTGTTGTCA d	28	0	0	-28	-28	154672	Methylene tetrahydrofolate dehydrogenase (NAD+ dependent), methenyltetrahydrofolate cyclohydrolase, reliable 3' end

Table 8. Genes differentially expressed in myoepithelial cells from DCIS and normal breast tissue

SEQ ID NO:	Tag_Sequence	NL	D6	D7	6/n	d7/n	Unigene	Gene
1485	TCCATCGTCC d	28	0	0	-28	-28	R34920	yg59g06.r1 Soares infant brain 1NIB Homo sapiens cDNA clone IMAGE:37058 5' similar to SP:CIKB_DROME P17970 POTASSIUM CHANNEL PROTEIN SHAB ;, mRNA sequence, undefined 3' end
1486	GTGCAGAGGA d	28	0	0	-28	-28	BE974249	601680217R2 NIH_MGC_83 Homo sapiens cDNA clone IMAGE:3950476 3', mRNA sequence, undefined 3'end
1487	GATATGTTAT d	28	0	0	-28	-28	117938	Collagen, type XVII, alpha 1, reliable 3' end
1488	ATGGTGTATG d	31	1	0	-28	-31	BE619862	601473114T1 NIH_MGC_68 Homo sapiens cDNA clone IMAGE:3876219 3', mRNA sequence, undefined 3' end
1489	TTACTTATAC d	63	2	0	-29	-63	C14491	C14491 Clontech human aorta polyA+ mRNA (#6572) Homo sapiens cDNA clone GEN-065B04 5', mRNA, undefined 3' end
1490	TTCTATTTCA d	32	1	0	-29	-32	170328	Moesin, reliable 3' end
1491	TGTTCATCAT d	35	1	2	-32	-15	65450	reticulon 4, reliable 3' end
1492	TGTTAATGTT d	35	1	2	-32	-15	261828	MAP kinase-interacting serine/threonine kinase 2, reliable 3' end
1493	TTTTGTATTT d	35	1	0	-32	-35	BF833948	RC1-HT0881-041100-019-a11 HT0881 Homo sapiens cDNA, mRNA sequence, undefined 3' end
1494	TCAATAAAGG d	32	0	0	-32	-32	118797	ubiquitin-conjugating enzyme E2D 3 (UBC4/5 homolog, yeast), reliable 3' end
1495	GTGATGGTGT d	37	1	2	-33	-15	197345	thyroid autoantigen 70kD (Ku antigen), reliable 3' end
1496	TCATCATCAG d	35	0	0	-35	-35	T94401	ye35f01.s1 Stratagene lung (#937210) Homo sapiens cDNA clone IMAGE:119737 3' similar to gb:M17886 60S ACIDIC RIBOSOMAL PROTEIN P1 (HUMAN);, mRNA sequence, undefined 3' end
1497	GGGAAGGGAC d	80	2	0	-36	-80	189559	EST, reliable 3' end
1498	GTAAATATGG d	124	3	0	-38	-124	198689	bullous pemphigoid antigen 1 (230/240kD), reliable 3' end
1499	TACCAGTGTA d	41	1	0	-38	-41	79037	heat shock 60kD protein 1 (chaperonin), reliable 3' end
1500	GTATTCTCCA d	38	0	0	-38	-38		No match
1501	TCCCCGTACA d	92	2	19	-42	-5		No match
1502	TACATAATTA d	48	1	2	-43	-20	240443	multiple endocrine neoplasia I, reliable 3' end
1503	TATGTGCACG d	44	0	0	-44	-44	AI874331	tz64c12.x1 NCI_CGAP_Ov35 Homo sapiens cDNA clone IMAGE:2293366 3' similar to TR:Q61402 Q61402 GRANULE CELL ANTISERUM POSITIVE 8 ;contains element LTR4 repetitive element ;, mRNA undefined 3' end
1504	TGATTGGTGG d	54	1	2	-49	-22	BQ374288	MR0-FT0176-040900-202-a01 FT0176 Homo sapiens cDNA, mRNA sequence, undefined 3' end
1505	TGCTTGTGTA d	52	0	0	-52	-52	BQ368670	PM3-GN0510-260501-010-f03 GN0510 Homo sapiens cDNA, mRNA sequence, undefined 3' end
1506	TATCTGTCTA d	60	1	0	-54	-60	145279	SET translocation (myeloid leukemia-associated), internally primed site
1507	ACCTTGGTGC d	61	1	0	-56	-61	R72649	yj95e04.s1 Soares breast 2NbHBst Homo sapiens cDNA clone IMAGE:156510 3' similar to gb:J00124_cds1 KERATIN, TYPE I CYTOSKELETAL 14 (HUMAN);, mRNA sequence, undefined 3' end
1508	TTTCCTTGCC d	63	0	0	-63	-63	AW070788	xa30d01.x1 NCI_CGAP_Br18 Homo sapiens cDNA clone IMAGE:2568289 3' similar to gb:Z19574_ma1KERATIN, TYPE I CYTOSKELETAL 17 (HUMAN);, mRNA sequence, reliable 3' end

Table 8. Genes differentially expressed in myoepithelial cells from DCIS and normal breast tissue

SEQ ID NO:	Tag_Sequence									Gene
		NL	D6	D7	6/n	d7/n	Unigene			
1509	ACACAGCAAG d	80	0	0	-80	-80	AW572695	xx92h01.x2 NCI_CGAP_Lym12 Homo sapiens cDNA clone IMAGE:2851153 3', mRNA sequence, reliable 3' end		
1510	TACTTTATAA d	127	1	0	-116	-127	8230	a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 1, reliable 3' end		

Table 9. Genes differentially expressed in luminal epithelial cells from DCIS and normal breast tissue

SEQ ID NO:	Tag_Sequence	NL	D6	D7	d6/n	d7/n	Unigene	Gene
1511	AGGAAGGAAC d	0	110	24	110	24	323910	V-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian), undefined 3' end
1512	GTAATCCTGC d	4	187	28	52	8	AW450286	UI-H-BI3-akz-e-09-0-ULs1 NCI_CGAP_Sub5 Homo sapiens cDNA clone IMAGE:2736089 3', mRNA, reliable 3' end
1513	GCTCAGCTGG d	0	31	16	31	16	223241	eukaryotic translation elongation factor 1 delta (guanine nucleotide exchange protein), reliable 3' end
1514	CCTGCCCACC d	0	21	15	21	15	1892	phenylethanolamine N-methyltransferase, reliable 3' end
1515	CCTGGCTAAT d	13	166	49	13	4	274170	Opa-interacting protein 2, reliable 3' end
1516	GCCCACAAGT d	2	22	46	12	25	285976	LAG1 longevity assurance homolog 2 (S. cerevisiae), reliable 3' end
1517	GGCAGCCAGA d	9	92	43	10	5	75061	Macrophage myristoylated alanine-rich C kinase substrate, reliable 3' end
1518	ACGCAGGGAG	11	99	77	9	7	279789	glucose phosphate isomerase, internal tag
1519	TTGGCCAGGA	11	89	38	8	3	46798	Homo sapiens mRNA; cDNA DKFZp434K152 (from clone DKFZp434K152), reliable 3' end
1520	TACCCTGGCA	4	28	23	8	6	AY014272	Homo sapiens FKSG30 (FKSG30) mRNA, shorter alternative transcript
1521	TCCCTATTAA	76	563	288	7	4	343430	ESTs, undefinded 3'end (NCBI only)
1522	GCTTTATTTG	62	365	226	6	4	288061	Actin, beta, reliable 3' end
1523	ACCCCCCCGC	64	372	364	6	6	2780	jun D proto-oncogene, undefined 3' end
1524	CACACAGTTT	15	70	71	5	5	204354	ras homolog gene family, member B, undefined 3' end
1525	AGGTCAGGAG	73	310	125	4	2	59498	Cell division cycle 2-like 5 (cholinesterase-related cell division controller), reliable 3' end
1526	TGGAAAGTGA	20	76	132	4	7	25647	v-fos FBJ murine osteosarcoma viral oncogene homolog, reliable 3' end
1527	GTGGCAGGCA	16	60	46	4	3	241205	Peroxisomal membrane protein 4 (24kD), reliable 3' end
1528	GCCTGCAGTC	13	45	81	4	6	31439	serine protease inhibitor, Kunitz type, 2, reliable 3' end
1529	ATGACCCCCG	13	44	42	3	3	AA918111	ol76d02.s1 NCI_CGAP_Kid3 Homo sapiens cDNA clone IMAGE:1535523 3', mRNA sequence, undefined 3' end
1530	CCTGTAGTCC	15	50	50	3	3	306226	Transmembrane gamma-carboxyglutamic acid protein 4, reliable 3' end
1531	ATCGTGGCGG d	42	105	972	3	23	5372	claudin 4, reliable 3' end
1532	CCTGTAATCC	152	353	292	2	2	292154	stromal cell protein (NCBI), reliable 3' end
1533	CCACTGCACT	125	275	194	2	2	107003	enhancer of invasion 10 (NCBI), reliable 3' end
1534	TGATTTCACT	294	441	865	2	3	X93334	mitochondria
1535	GTGTGGGGGG	54	18	21	-3	-3	2340	Junction plakoglobin, reliable 3' end
1536	ATTCTCCAGT	87	28	22	-3	-4	234518	ribosomal protein L23, reliable 3' end
1537	GCCGTGTCCG	258	82	58	-3	-4	350166	ribosomal protein S6, reliable 3' end
1538	CAGCTCACTG	58	18	17	-3	-3	738	ribosomal protein L14, reliable 3' end

Table 9. Genes differentially expressed in luminal epithelial cells from DCIS and normal breast tissue

SEQ ID NO:	Tag_Sequence	NL	D6	D7	d6/n	d7/n	Unigene	Gene
1539	GCCTGTATGA	67	21	20	-3	-3	180450	ribosomal protein S24, reliable 3' end
1540	CTGCCAACTT	56	17	22	-3	-3	180370	cofilin 1 (non-muscle), internal tag
1541	CAAGTTTGCT d	36	11	3	-3	-12	181165	eukaryotic translation elongation factor 1 alpha 1, internal tag
1542	GGGCTGGGGT	267	78	74	-3	-4	90436	Sperm associated antigen 7, reliable 3' end
1543	CGCCGCCGGC	281	76	97	-4	-3	182825	ribosomal protein L35, reliable 3' end
1544	GTAAAAAAAA	64	17	18	-4	-4	460	Activating transcription factor 3, reliable 3' end
1545	TAGAAAGGCA	36	10	6	-4	-6	U07802	Human Tis11d gene, reliable 3' end
1546	TGAAATAAAA	87	23	21	-4	-4	9614	nucleophosmin (nucleolar phosphoprotein B23, numatrin), reliable 3' end
1547	TGAAAAAAAA	33	9	7	-4	-5	119178	Cation-chloride cotransporter-interacting protein, reliable 3' end
1548	ACTCCAAAAA	158	40	48	-4	-3	BC012990	Homo sapiens, clone IMAGE:3840457, mRNA, reliable 3' end
1549	TGGAAGCACT d	368	94	15	-4	-25	624	interleukin 8, reliable 3' end
1550	GATGAACTGA	29	7	6	-4	-5	30035	Splicing factor, arginine/serine-rich 10 (transformer 2 homolog, Drosophila), reliable 3' end
1551	GCCGCCCTGC	132	33	18	-4	-7	82208	acyl-Coenzyme A dehydrogenase, very long chain, reliable 3' end
1552	AGAAAAAAAA	83	21	20	-4	-4	597	Glutamic-oxaloacetic transaminase 1, soluble (aspartate aminotransferase 1), reiable 3' end
1553	CCCCAGCCAG	143	35	33	-4	-4	252259	Ribosomal protein S3, reliable 3' end
1554	TTGAAGCTTT d	122	29	5	-4	-24	75765	GRO2 oncogene, reliable 3' end
1555	AGCTCTCCCT	107	26	47	-4	-2	82202	ribosomal protein L17, reliable 3' end
1556	CAAAAAAAAA	107	24	22	-4	-5	1217	Adenosine deaminase, reliable 3' end
1557	CCCATCCGAA	112	26	23	-4	-5	91379	ribosomal protein L26, reliable 3' end
1558	AGGGGCGCAG	38	9	11	-4	-3	97616	SH3-domain GRB2-like 1, reliable 3' end
1559	GTCTGCACCT	33	7	8	-4	-4	376798	Homo sapiens mRNA; cDNA DKFZp547C162 (from clone DKFZp547C162), reliable 3' end
1560	CCAGAACAGA	123	27	59	-5	-2	334807	Ribosomal protein L30, reliable 3' end
1561	GTGTTAACCA	58	12	20	-5	-3	74267	ribosomal protein L15, shorter alternative transcript
1562	CTGGGTTAAT	299	62	97	-5	-3	298262	ribosomal protein S19, reliable 3' end
1563	GTCTTAAAGT d	100	21	8	-5	-12	177781	Homo sapiens, clone IMAGE:4711494, mRNA, reliable 3' end
1564	AGAGAAATTT	54	11	13	-5	-4	77028	SEC61B Protein translocation complex beta, reliable 3' end
1565	CTTCGAAACT	67	13	12	-5	-6	51299	NADH dehydrogenase (ubiquinone) flavoprotein 2 (24kD), reliable 3' end
1566	TTGGTCCTCT	435	87	185	-5	-2	356795	ribosomal protein L41, reliable 3' end
1567	TGCACGTTTT	490	97	96	-5	-5	169793	ribosomal protein L32, reliable 3' end
1568	GTGCGCTGAG	103	20	56	-5	-2	277477	HLA-C Major histocompatibility complex, class I, C, reliable 3' end
1569	GGGAAGCAGA	78	15	158	-5	0	X93334	mitochondria

Table 9. Genes differentially expressed in luminal epithelial cells from DCIS and normal breast tissue

SEQ ID NO:	Tag_Sequence	NL	D6	D7	d6/n	d7/n	Unigene	Gene
1570	GCATAATAGG	82	15	35	-6	-2	350077	ribosomal protein L21, reliable 3' end
1571	GAAATAAAGT	27	5	4	-6	-7	26498	hypothetical protein FLJ21657, short alternative transcript
1572	CAACTAATTC	116	21	40	-6	-3	75106	clusterin (complement lysis inhibitor, SP-40,40, sulfated glycoprotein 2, testosterone-repressed prostate message 2, apolipoprotein J), reliable 3' end
1573	GCTGCCCTTG	103	18	32	-6	-3	348557	tubulin alpha 6, reliable 3' end
1574	GTTTATGGAT d	111	20	1	-6	-111	365706	matrix Gla protein, reliable 3' end
1575	AATAGGTCCA	132	23	34	-6	-4	113029	ribosomal protein S25, reliable 3' end
1576	CTTCCTGTGA d	494	82	5	-6	-99	348419	LOC118430 Small breast epithelial mucin, undefined 3' end
1577	AACTAAAAAA	111	18	9	-6	-12	3297	ribosomal protein S27a, reliable 3' end
1578	CCCCCTGGAT	60	10	12	-6	-5	275243	S100 calcium binding protein A6 (calcyclin), reliable 3' end
1579	GGCACCTCAG	31	5	6	-6	-5	93913	interleukin 6 (interferon, beta 2), reliable 3' end
1580	TAAGGAGCTG	125	20	67	-6	-2	299465	ribosomal protein S26, reliable 3' end
1581	TTGAAACTTT d	394	61	1	-6	-394	789	GRO1 oncogene (melanoma growth stimulating activity, alpha), reliable 3' end
1582	TTGGCCAGGG d	111	17	10	-6	-11	321687	F-box protein FBX30, reliable 3' end
1583	TAAAAAAAAA	64	10	14	-6	-5	77910	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble) (reliable 3'end to this and several others)
1584	CAATAAACTG	103	16	31	-7	-3	150580	putative translation initiation factor, shorter alternative transcript
1585	TTTGAAATGA	129	20	55	-7	-2	28491	spermidine/spermine N1-acetyltransferase, reliable 3' end
1586	CACAAACGGT	218	33	109	-7	-2	195453	ribosomal protein S27 (metallopanstimulin 1), reliable 3' end
1587	AAGGAGATGG	98	15	31	-7	-3	164170	vascular Rab-GAP/TBC-containing, reliable 3' end
1588	GTGACCACGG	132	20	58	-7	-2	BQ447386	UI-H-EU1-bae-f-07-0-UI.s1 NCI_CGAP_Ct1 Homo sapiens cDNA clone UI-H-EU1-bae-f-07-0-UI 3'mRNA, reliable 3' end
1589	TAATAAAGGT	42	6	11	-7	-4	151604	ribosomal protein S8, reliable 3' end
1590	CTCACTTTTT	154	22	22	-7	-7	76722	CCAAT/enhancer binding protein (C/EBP), delta, reliable 3' end
1591	TTCACTGTGA d	34	5	3	-7	-11	621	lectin, galactoside-binding, soluble, 3 (galectin 3), reliable 3' end
1592	CTTCCTTGCC	27	4	6	-7	-5	2785	keratin 17, reliable 3' end
1593	GTGAAAAAAA	36	5	4	-7	-9	352394	Hypothetical protein BC013113, reliable 3' end
1594	TGACTGGCAG	49	6	9	-8	-5	278573	CD59 antigen p18-20 (antigen identified by monoclonal antibodies 16.3A5, EJ16, EJ30, EL32 and G344), reliable 3'end, similarity to urokinase plasminogen activator receptor
1595	AATGAGCAAC	20	2	3	-8	-7	171862	guanylate binding protein 2, interferon-inducible, shorter alternative transcript
1596	GTGGAGCGGA d	20	2	2	-8	-10	323462	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 30, reliable 3' end
1597	CCATTGAAAC d	20	2	0	-8	-20	75517	laminin, beta 3 (nicein (125kD), kalinin (140kD), BM600 (125kD)), reliable 3' end
1598	GAAAACAAAG d	20	2	1	-8	-20	99936	keratin 10 (epidermolytic hyperkeratosis; keratosis palmaris et plantaris), reliable 3' end
1599	TTGGCTTTTC	31	4	4	-8	-8	41569	phosphatidic acid phosphatase type 2A, internally primed site
1600	TAAAAACTTT d	62	7	4	-8	-15	204096	secretoglobin, family 1D, member 2, reliable 3' end

Table 9. Genes differentially expressed in luminal epithelial cells from DCIS and normal breast tissue

SEQ ID NO:	Tag_Sequence	NL	D6	D7	d6/n	d7/n	Unigene	Gene
1601	TCGCCGCGAC	22	2	4	-9	-5	296290	ribosomal protein L37a, undefined 3' end
1602	CAGGCCCCAC d	47	5	11	-10	-4	256290	S100 calcium binding protein A11 (calgizzarin), reliable 3' end
1603	AGCAGATCAG d	189	20	37	-10	-5	119301	S100 calcium binding protein A10 (annexin II ligand, calpactin I, light polypeptide (p11)), reliable 3' end
1604	ATAATAAAAG d	24	2	0	-10	-24	89690	GRO3 oncogene, reliable 3' end
1605	AGAAAGATGT d	83	9	4	-10	-21	78225	annexin A1 reliable 3' end
1606	GCGACAGCTC d	36	4	8	-10	-5	BE719410	CM2-HT0847-050800-313-c12 HT0847 Homo sapiens cDNA, mRNA sequence, undefined 3' end
1607	TGCTAATTGT d	25	2	6	-10	-4	71968	Homo sapiens mRNA; cDNA DKFZp564F053 (from clone DKFZp564F053), reliable 3' end
1608	GCAACTTAGA d	29	2	1	-12	-29	54451	LAMC2 Laminin, gamma 2 (nicein (100kD), kalinin (105kD), BM600 (100kD), Herlitz junctional epidermolysis bullosa)) shorter alternative transcript
1609	TCCCCGTACA d	439	37	98	-12	-4		no match
1610	CGTGGGTGGG d	74	6	0	-12	-74	202833	Heme oxygenase (decycling) 1, reliable 3' end
1611	TGCAGTGACT d	13	0	0	-13	-13	79691	LIM domain protein, reliable 3' end
1612	TGCAAACAGC d	13	0	0	-13	-13	BF675978	602083935F1 NIH_MGC_83 Homo sapiens cDNA clone IMAGE:4248177 5', mRNA sequence, internal tag
1613	GGGTGGGCAG d	13	0	0	-13	-13	284226	F-box only protein 6, reliable 3' end
1614	CTGAAAATTG d	13	0	0	-13	-13	106880	bystin-like, reliable 3' end
1615	AGGTGTGAGC d	13	0	0	-13	-13	323767	ESTs, internal tag
1616	AGCAGTGACG d	13	0	0	-13	-13	116651	epithelial V-like antigen 1, reliable 3' end
1617	AGAATTTAGG d	13	0	0	-13	-13	105094	ESTs, undefined 3' end
1618	TCTGGGGACG d	16	1	1	-13	-16	12163	eukaryotic translation initiation factor 2, subunit 2 (beta, 38kD, internally primed site
1619	GTACTAGTGT d	33	2	1	-13	-33	303649	small inducible cytokine A2 (monocyte chemotactic protein 1), reliable 3' end
1620	CGAATGTCCT d	53	4	0	-14	-53	335952	keratin 6B, reliable 3' end
1621	GCTCAAAAAC d	15	0	0	-15	-15	R92600	yq07f04.s1 Soares fetal liver spleen 1NFLS Homo sapiens cDNA clone IMAGE:196255 3' similar to contains Alu repetitive element;, mRNA sequence, undefined 3' end
1622	CCCGCCTCTT d	15	0	0	-15	-15	BQ358365	IL3-HT0617-280800-258-G06 HT0617 Homo sapiens cDNA, mRNA sequence, undefined 3' end
1623	ACAGGAAACT d	15	0	0	-15	-15	69149	proline-serine-threonine phosphatase interacting protein 2, reliable 3' end
1624	TAATTTTGGA d	15	0	1	-15	-15	292457	Homo sapiens, clone MGC:16362 IMAGE:3927795, mRNA, complete cds, reliable 3' end
1625	AAGCTCGCCG d	125	9	0	-15	-125	62492	secretoglobin, family 3A, member 1, reliable 3' end
1626	GACTCTTCAG d	396	27	119	-15	-3	234726	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3, reliable 3' end
1627	GAGCAGCGCC d	18	1	2	-15	-9	112408	S100 calcium binding protein A7 (psoriasin 1), reliable 3' end

Table 9. Genes differentially expressed in luminal epithelial cells from DCIS and normal breast tissue

SEQ ID NO:	Tag_Sequence	NL	D6	D7	d6/n	d7/n	Unigene	Gene
1628	CTTCAAAAAA d	18	1	1	-15	-18	6126	Mannosidase, beta A, lysosomal-like, reliable 3' end
1629	CTAAAAAAAA d	38	2	8	-16	-5	54457	CD81 antigen (target of antiproliferative antibody 1), reliable 3' end
1630	GGTGAGTTAC d	16	0	0	-16	-16	118183	hypothetical protein FLJ22833, internally primed site
1631	GTGGTTAAAA d	20	1	0	-16	-20	99949	Prolactin-induced protein, internal tag
1632	CCCGAGGCAG d	62	4	4	-17	-15	155223	stanniocalcin 2, reliable 3' end
1633	GCCTTGGGTG d	64	4	10	-17	-6	2250	leukemia inhibitory factor (cholinergic differentiation factor), internal tag
1634	GACAAAAAAA d	44	2	11	-18	-4	32366	DERMO1 Likely ortholog of mouse and rat twist-related bHLH protein Dermo-1, reliable 3' end
1635	GGGAAGGCAC d	22	1	3	-18	-7	13144	ORM1-like 2 (S. cerevisiae), reliable 3' end
1636	GAGGGTTTAG d	44	2	2	-18	-22	75498	small inducible cytokine subfamily A (Cys-Cys), member 20, reliable 3' end
1637	GCGCGATGCA d	18	0	2	-18	-9	AI420761	te91a02.x1 NCI_CGAP_Pr28 Homo sapiens cDNA clone IMAGE:2094026 3', mRNA sequence, undefined 3' end
1638	TTGAATCCCC d	18	0	0	-18	-18	112341	protease inhibitor 3, skin-derived (SKALP), reliable 3' end
1639	GACACGAACA d	45	2	2	-19	-23	25829	RAS, dexamethasone-induced 1, reliable 3' end
1640	GCGGCTTTCC d	51	2	15	-21	-3	278431	SCO cytochrome oxidase deficient homolog 2 (yeast), reliable 3' end
1641	GCTTGCAAAA d	210	10	3	-22	-70	372783	superoxide dismutase 2, mitochondrial, reliable 3' end
1642	GTGTGGCAGC d	22	0	0	-22	-22	42676	KIAA0781 protein, undefined 3' end
1643	TTTTGTGTGA d	27	1	4	-22	-7	182698	mitochondrial ribosomal protein L20, undefined 3' end
1644	CTGGCCCTCG d	296	12	74	-24	-4	350470	Trefoil factor 1 (breast cancer, estrogen-inducible sequence expressed in), reliable 3' end
1645	AGGTCTGCCA d	27	0	5	-27	-5	201967	aldo-keto reductase family 1, member C2 (dihydrodiol dehydrogenase 2; bile acid binding protein; 3-alpha hydroxysteroid dehydrogenase, type III), reliable 3' end
1646	TCTCCAACAA d	27	0	0	-27	-27	T69914	yc19b07.s1 Stratagene lung (#937210) Homo sapiens cDNA clone IMAGE:81109 3' similar to gb:J03600 ARACHIDONATE 5-LIPOXYGENASE (HUMAN);, mRNA sequence, undefined 3' end
1647	GGTAAAATTA d	29	0	2	-29	-15	340959	Ts translation elongation factor, mitochondrial, reliable 3' end
1648	CTTAAAAAAA d	36	1	0	-30	-36	75063	human immunodeficiency virus type I enhancer binding protein 2, reliable 3' end
1649	GCAGGCCAAG d	93	2	16	-38	-6	69771	B-factor, properdin, reliable 3' end
1650	GGAAAAGTGG d	96	2	2	-39	-48	297681	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1, reliable 3' end
1651	TTTGCTTTTG d	40	0	8	-40	-5	234642	aquaporin 3, reliable 3' end
1652	CTTCTCCAAA d	42	0	0	-42	-42	W03794	za61g08.r1 Soares fetal liver spleen 1NFLS Homo sapiens cDNA clone IMAGE:297086 5' similar to gb:X54486_ma1 PLASMA PROTEASE C1 INHIBITOR PRECURSOR (HUMAN);, mRNA, undefined 3' end
1653	TTGGTTTTTG d	56	1	0	-46	-56	164021	Small inducible cytokine subfamily B (Cys-X-Cys), member 6 (granulocyte chemotactic protein 2), reliable 3' end
1654	GTGCGGAGGA d	60	0	1	-60	-60	332053	serum amyloid A1, reliable 3' end

Table 9. Genes differentially expressed in luminal epithelial cells from DCIS and normal breast tissue

SEQ ID NO:	Tag_Sequence	NL	D6	D7	d6/n	d7/n	Unigene	Gene
1655	TGCAGCACGA d	67	0	6	-67	-11	277477	HLA-C Major histocompatibility complex, class I, C, reliable 3' end
1656	ACACAGCAAG d	243	0	0	-243	-243	AW57269 5	xx92h01.x2 NCI_CGAP_Lym12 Homo sapiens cDNA clone IMAGE:2851153 3', mRNA sequence, reliable 3' end

Table. 10 Genes differentially expressed in endothelial cells from DCIS and normal breast tissue

SEQ ID NO:	Tag_Sequence	NL	D6	d6/n	Unigene	Gene
1657	CGTGGGTGGG d	0	73	73	202833	Heme oxygenase (decycling) 1, reliable 3' end
1658	TTTGAGGATT d	0	33	33	18792	thioredoxin-like, 32kD, internal tag
1659	TAAATAATTT d	0	33	33	1197	heat shock 10kD protein 1 (chaperonin 10), reliable 3' end
1660	GCAGAATAGA d	0	29	29	236218	Tripartite motif-containing 32, internal tag
1661	GATAACTACA d	0	27	27	119206	insulin-like growth factor binding protein 7, shorter alternative transcript
1662	GCTTTCTCAC d	0	26	26	BG223065	nah42g11.x1 NCI_CGAP_HN21 Homo sapiens cDNA clone IMAGE:4233812 3', mRNA sequence, undefined 3' end
1663	GAAAAGGTTA d	0	22	22	16085	putative G-protein coupled receptor, reliable 3' end
1664	AAATTGTTGG d	0	22	22	120932	ESTs, reliable 3' end
1665	GTAATGACAG d	0	21	21	25590	stanniocalcin 1, reliable 3' end
1666	TGCCTCTGTC d	0	21	21	AA954388	oo01c02.s1 Soares_NFL_T_GBC_S1 Homo sapiens cDNA clone IMAGE:1564898 3' similar to gb:X00737 PURINE NUCLEOSIDE PHOSPHORYLASE (HUMAN);, mRNA sequence, reliable 3' end
1667	TCTTGATTTA d	0	21	21	74561	alpha-2-macroglobulin, reliable 3' end
1668	GACGACTGAC d	0	21	21	155530	interferon, gamma-inducible protein 16, reliable 3' end
1669	CCCCCTGCCC d	3	40	15	177596	Hypothetical protein FLJ10350, reliable 3' end
1670	CAGTTCTCTG d	3	38	15	279921	hypothetical protein MGC8721, reliable 3' end
1671	AGACAAGCTG d	3	37	14	166975	Splicing factor, arginine/serine-rich 5, reliable 3' end
1672	ACAGTGGGGA d	3	37	14	278270	Unactive progesterone receptor, 23 kD, reliable 3' end
1673	CCTGTGTTGG d	5	71	14	AV728954	AV728954 HTC Homo sapiens cDNA clone HTCCGG11 5', mRNA sequence, internal tag
1674	ATGTCTTTTC d	3	34	13	1516	insulin-like growth factor binding protein 4, undefined 3' end
1675	CATTTCAGAG d	3	32	12	15259	BCL2-associated athanogene 3, reliable 3' end
1676	GGATTGTCTG d	3	30	12	83753	small nuclear ribonucleoprotein polypeptides B and B1, reliable 3' end
1677	TTAGTGTCGT d	3	27	11	AW805523	QV1-UM0103-250400-173-f02 UM0103 Homo sapiens cDNA, mRNA sequence, undefined 3' end
1678	AGGAACTGTA d	3	27	11	184634	hypothetical protein FLJ20005, reliable 3' end
1679	ACAGCGCTGA d	3	27	11	352392	major histocompatibility complex, class II, DR beta 5
1680	GGCTGGTCTG d	10	108	10	337986	hypothetical protein MGC4677, reliable 3' end
1681	GACCGCAGGA d	16	161	10	119129	collagen, type IV, alpha 1, reliable 3' end
1682	TAATTTGCAT d	5	54	10	79368	epithelial membrane protein 1, reliable 3' end
1683	AAAACATTCT d	117	1175	10	X93334	mitochondrial
1684	TCTCTGAGCA	5	38	7	211604	a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 4, reliable 3' end
1685	TTTAACGGCC	36	268	7	X93334	mitochondrial

Table. 10 Genes differentially expressed in endothelial cells from DCIS and normal breast tissue

SEQ ID NO:	Tag_Sequence	NL	D6	d6/n	Unigene	Gene
1686	TGTACCTGTA	8	56	7	334842	Tubulin, alpha, ubiquitous, reliable 3' end
1687	TCCAGAATCC	8	56	7	7764	KIAA0469 gene product, reliable 3' end
1688	GGAAGGGGAG	5	37	7	73090	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100), reliable 3' end
1689	AAAACTGCAC	5	37	7	8084	hypothetical protein dJ465N24.2.1, reliable 3' end
1690	CATATCATTA	42	277	7	119206	insulin-like growth factor binding protein 7, reliable 3' end
1691	AGACCAAAGT	13	86	7	82646	DnaJ (Hsp40) homolog, subfmaily B, member 1, reliable 3' end
1692	TGTAGTTTGA	5	33	6	171626	transcription elongation factor B (SIII), polypeptide 1-like, reliable 3' end
1693	TGCTGTGCAT	10	60	6	75692	Asparagine synthetase, reliable 3' end
1694	TATGAGGGTA	8	45	6	24950	regulator of G-protein signalling 5, reliable 3' end
1695	GCCATAAAAT	8	45	6	1908	proteoglycan 1, secretory granule, reliable 3' end
1696	AAGACAGTGG	21	118	6	296290	Ribosomal protein L37a, reliable 3' end
1697	CCAATTTATC	8	44	6	94	DnaJ (Hsp40) homolog, subfamily A, member 1, reliable 3' end
1698	AAAGTGAAGA	8	41	5	334477	FLJ23277 protein, reliable 3' end
1699	CCAGGAGGAA	18	95	5	180414	heat shock 70kD protein 8, reliable 3' end
1700	GAGAACCGTA	8	40	5	105547	neural proliferation, differentiation and control, 1, reliable 3' end
1701	TGTTCTGGAG	10	52	5	74471	Gap junction protein, alpha 1, 43kD (connexin 43), reliable 3' end
1702	AAGGAGATGG	18	91	5	164170	vascular Rab-GAP/TBC-containing, reliable 3' end
1703	TGTCCTGGTT	26	129	5	179665	Cyclin-dependent kinase inhibitor 1A (p21, Cip1), reliable 3' end
1704	GGAGAGGAAG	8	38	5	16313	Kruppel-like zinc finger protein GLIS2, reliable 3' end
1705	CTGACCTGTG	26	126	5	BM151142	TCBAP1D13652 Pediatric pre-B cell acute lymphoblastic leukemia Baylor-HGSC project=TCBA Homo sapiens cDNA clone TCBAP1365, mRNA sequence, reliable 3' end
1706	TGGAAGCACT	23	113	5	624	interleukin 8, reliable 3' end
1707	CACAAACGGT	94	431	5	195453	ribosomal protein S27 (metallopanstimulin 1), reliable 3' end
1708	AAGGGAGGGT	18	80	4	182248	sequestosome 1, reliable 3' end
1709	TAACAGCCAG	31	130	4	81328	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha, reliable 3' end
1710	ACATCATCGA	18	76	4	182979	ribosomal protein L12, reliable 3' end
1711	GTGACCACGG	10	43	4	BQ447386	UI-H-EU1-bae-f-07-0-ULs1 NCI_CGAP_Ct1 Homo sapiens cDNA clone UI-H-EU1-bae-f-07-0-UI 3' mRNA, reliable 3' end
1712	TGTTGAAAAA	10	43	4	89546	selectin E (endothelial adhesion molecule 1), reliable 3' end
1713	GTTCACTGCA	16	63	4	168383	intercellular adhesion molecule 1 (CD54), human rhinovirus receptor, reliable 3' end
1714	CCAGAACAGA	49	198	4	334807	ribosomal protein L30, reliable 3' end
1715	CTCATAAGGA	18	73	4	X93334	mitochondrial
1716	CTTAATCCTG	16	60	4	298275	solute carrier family 38, member 2, reliable 3' end

Table. 10 Genes differentially expressed in endothelial cells from DCIS and normal breast tissue

SEQ ID NO:	Tag_Sequence	NL	D6	d6/n	Unigene	Gene
1717	TTTGAAATGA	18	70	4	28491	spermidine/spermine N1-acetyltransferase, reliable 3' end
1718	ATAATTCTTT	104	397	4	539	ribosomal protein S29, reliable 3' end
1719	AGATTCAAAC	13	49	4	14368	SH3 domain binding glutamic acid-rich protein like
1720	CCGTCCAAGG	44	166	4	80617	ribosomal protein S16, reliable 3' end
1721	TAATCCTCAA	18	62	3	78409	collagen, type XVIII, alpha 1, shorter alternative transcript
1722	GTGCGCTGAG	44	150	3	277477	Major histocompatibility complex, class I, C, reliable 3' end
1723	GTTCCCTGGC	21	69	3	177415	Finkel-Biskis-Reilly murine sarcoma virus (FBR-MuSV) ubiquitously expressed (fox derived); ribosomal protein S30, reliable 3' end
1724	TGAAGTAACA	18	59	3	150580	putative translation initiation factor, reliable 3' end
1725	CCTAGCTGGA	36	117	3	342389	peptidylprolyl isomerase A (cyclophilin A), reliable 3' end (intracellular receptor)
1726	TACCATCAAT	18	58	3	169476	glyceraldehyde-3-phosphate dehydrogenase, reliable 3' end
1727	AATCCTGTGG	18	58	3	178551	ribosomal protein L8, reliable 3' end
1728	CAGAGATGAA	57	181	3	8997	Sad1 unc-84 domain protein 1, reliable 3' end
1729	AAGGTGGAGG	55	170	3	163593	Ribosomal protein L18a, reliable 3' end
1730	TGCACTTCAA	52	155	3	75445	SPARC-like 1 (mast9, hevin), reliable 3' end
1731	GGCCTGCTGC	21	62	3	9634	LOC113246 Hypothetical protein BC009925, reliable 3' end
1732	AGGGCTTCCA	76	218	3	29797	ribosomal protein L10, shorter alternative transcript
1733	GTGAAGGCAG	60	173	3	77039	ribosomal protein S3A, reliable 3' end
1734	CAAGCATCCC	65	187	3	X93334	mitochondrial
1735	AGAATCACTT	26	73	3	130815	hypothetical protein FLJ21870, reliable 3' end
1736	GAAGCAGGAC	34	92	3	180370	cofilin 1 (non-muscle), reliable 3' end
1737	GCTTTTAAGG	36	99	3	8102	Ribosomal protein S20, reliable 3' end
1738	GCATAATAGG	68	181	3	350077	ribosomal protein L21, reliable 3' end
1739	CCCTGGGTTC	29	73	3	111334	Ferritin, light polypeptide, reliable 3' end
1740	GGGACGAGTG	68	169	2	351316	Transmembrane 4 superfamily member 1, reliable 3' end
1741	GGCAAGAAGA	36	89	2	111611	ribosomal protein L27, reliable 3' end
1742	TGTGCTAAAT	34	82	2	250895	ribosomal protein L34, shorter alternative transcript
1743	ATGTGAAGAG	180	432	2	111779	secreted protein, acidic, cysteine-rich (osteonectin), reliable 3' end
1744	TCAGATCTTT	109	259	2	108124	ribosomal protein S4, X-linked, reliable 3' end
1745	CTAAGACTTC	380	885	2	X93334	mitochondrial
1746	CAATAAATGT	60	137	2	337445	ribosomal protein L37, reliable 3' end
1747	GTTGTGGTTA	219	493	2	75415	beta-2-microglobulin, reliable 3' end
1748	GGATTTGGCC	182	393	2	351937	Ribosomal protein, large P2, reliable 3' end
1749	GTGCTGAATG	52	111	2	77385	Myosin, light polypeptide 6, alkali, smooth muscle and non-muscle, reliable 3' end

Table. 10 Genes differentially expressed in endothelial cells from DCIS and normal breast tissue

SEQ ID NO:	Tag_Sequence	NL	D6	d6/n	Unigene	Gene
1750	GGAGTGTGCT	57	114	2	9615	myosin, light polypeptide 9, regulatory, reliable 3' end
1751	GGCAAGCCCC	86	166	2	334895	ribosomal protein L10a, reliable 3' end
1752	TAGGTTGTCT	169	327	2	279860	Tumor protein, translationally-controlled 1, reliable 3' end
1753	TTGGTCCTCT	180	346	2	356795	ribosomal protein L41, reliable 3' end
1754	TCCAAATCGA	120	218	2	297753	vimentin, reliable 3' end
1755	CTGGGTTAAT	177	318	2	298262	ribosomal protein S19, reliable 3' end
1756	TGGAAAGTGA	175	313	2	25647	v-fos FBJ murine osteosarcoma viral oncogene homolog, reliable 3' end
1757	TGGTGTTGAG	94	165	2	275865	ribosomal protein S18, reliable 3' end
1758	GCCGAGGAAG	112	196	2	339696	ribosomal protein S12, reliable 3' end
1759	CACCTAATTG	175	299	2	X93334	mitochondrial
1760	GAAAAATGGT	117	191	2	181357	laminin receptor 1 (67kD, ribosomal protein SA), reliable 3' end
1761	TGCACGTTTT	234	379	2	169793	ribosomal protein L32, reliable 3' end
1762	GGGCTGGGGT	180	288	2	90436	Sperm associated antigen 7, reliable 3' end
1763	AGCACCTCCA	133	211	2	75309	eukaryotic translation elongation factor 2, reliable 3' end
1764	ACCAAAAACC	201	51	-2	172928	collagen, type I, alpha 1, internally primed site
1765	CAAATCCAAA	55	14	-2	227400	mitogen-activated protein kinase kinase kinase kinase 3
1766	TTACCATATC	44	11	-2	300141	ribosomal protein L39
1767	GAAATAAAGC	52	12	-2	300697	immunoglobulin heavy constant gamma 3 (G3m marker), reliable 3' end
1768	ACCCCCCCGC	656	147	-2	2780	jun D proto-oncogene, undefined 3' end
1769	CGAGGGGCCA	39	8	-3	182485	actinin, alpha 4, undefined 3' end
1770	GATCAGGCCA	120	25	-3	119571	Collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant), shorter alternative transcript
1771	TTTCCCTCAA	34	7	-3	75111	protease, serine, 11 (IGF binding), similar to IGFBP7, cleaves IGF
1772	GAGCAGCTGG	31	5	-3	166887	copine I, reliable 3' end
1773	TTTGCACCTT	120	21	-3	75511	connective tissue growth factor, undefined 3' end
1774	AGCCACCGCG	47	7	-4	193716	Complement component (3b/4b) receptor 1, including Knops blood group system, reliable 3' end
1775	GGCCGCGAGG	47	7	-4	78344	myosin, heavy polypeptide 11, smooth muscle, internally primed site
1776	GGGGTAAGAA	29	4	-4	80423	prostatic binding protein, reliable 3' end
1777	GGCCCGGCTT	29	4	-4	283639	chromosome 2 open reading frame 9, reliable 3' end
1778	GGGCCAACCC	65	8	-4	BI012736	PM3-ET0153-100101-008-c01 ET0153 Homo sapiens cDNA, mRNA sequence, undefined 3' end
1779	GACCAGCAGA	34	4	-4	172928	Collagen, type I, alpha 1, internal tag
1780	CTAAAATAGT	39	4	-5	93557	proenkephalin (NCBI only)
1781	GGCAATTCAA	26	3	-5	349150	Homo sapiens cDNA FLJ33107 fis, clone TRACH2000959, reliable 3' end

Table. 10 Genes differentially expressed in endothelial cells from DCIS and normal breast tissue

SEQ ID NO:	Tag_Sequence	NL	D6	d6/n	Unigene	Gene
1782	CCCCGCCAAG	26	3	-5	169718	Calponin 2, reliable 3' end
1783	TCCCTATTAG	16	0	-6		no match
1784	GCCAAAACCT	16	0	-6	158287	syndecan 3 (N-syndecan
1785	CCCCTATTAA	16	0	-6		no match
1786	GGGGGCTCAG	31	3	-6	276919	ESTs, reliable 3' end
1787	GAGATCCGCA	31	3	-6	75348	proteasome (prosome, macropain) activator subunit 1 (PA28 alpha), reliable 3' end
1788	GCCGGCTCAT	16	0	-6	AA213605	zq93d11.r1 Stratagene hNT neuron (#937233) Homo sapiens cDNA clone IMAGE:649557 5' similar to contains Alu repetitive element;, mRNA sequence, undefined 3' end
1789	GATTCTGGGT	16	0	-6	334637	MGC15619 Hypothetical protein MGC15619, internal tag
1790	ACACAGCAAG	125	10	-7	AW572695	xx92h01.x2 NCI_CGAP_Lym12 Homo sapiens cDNA clone IMAGE:2851153 3', mRNA sequence, reliable 3'end
1791	CTCAACCCCC	36	3	-7	89137	Low density lipoprotein-related protein 1 (alpha-2-macroglobulin receptor), reliable 3'end
1792	CTCTCAATAT	18	0	-7	279518	amyloid beta (A4) precursor-like protein 2, shorter alternative transcript
1793	CCCGCCTCTT	18	0	-7	BQ358365	IL3-HT0617-280800-258-G06 HT0617 Homo sapiens cDNA, mRNA sequence, undefined 3' end
1794	GGGGTGCTGT	18	0	-7	166161	dynamin 1, reliable 3' end
1795	GCTAGGCCGG	18	0	-7	BG876456	QV0-DT0020-090200-106-b04 DT0020 Homo sapiens cDNA, mRNA sequence, undefined 3' end
1796	GAGCCAGGCT	18	0	-7	83326	matrix metalloproteinase 3 (stromelysin 1, progelatinase), reliable 3' end
1797	AGGGTCCCCG	18	0	-7	Z00013	H.sapiens germline gene for the leader peptide and variable region of a kappa immunoglobulin (subgroup V kappa I, undefined 3' end
1798	TGGCTGGGAA	21	1	-8	172684	vesicle-associated membrane protein 8 (endobrevin), reliable 3' end
1799	GAGAGAAAAT	21	1	-8	181444	Hypothetical protein LOC51235, reliable 3' end
1800	CCTGTGGTCC	21	1	-8	334541	Similar to Zinc finger protein 20 (Zinc finger protein KOX13), reliable 3' end
1801	CCTCCAGCTA	21	1	-8	242463	keratin 8, reliable 3' end
1802	ATCAAATCCA	21	1	-8	288581	Homo sapiens mRNA for FLJ00239 protein, internal tag
1803	GTCAAAATTT	21	0	-8	108623	Thrombospondin 2, reliable 3' end
1804	GAAACCCCAG	21	0	-8	84359	Likely ortholog of Xenopus dullard, reliable 3' end
1805	CTCCACCCGA	21	0	-8	311815	EST, reliable 3' end
1806	TTAAATAGCA	21	1	-8	76698	stress-associated endoplasmic reticulum protein 1; ribosome associated membrane protein 4, internally primed site
1807	CTAACGGGGC	21	1	-8	102171	immunoglobulin superfamily containing leucine-rich repeat, reliable 3' end
1808	GTGCTAAGCA	21	0	-8	AI811424	tw73h08.x1 NCI_CGAP_Ut3 Homo sapiens cDNA clone IMAGE:2265375 3' similar to S W:CA26_MOUSE Q02788 COLLAGEN ALPHA 2(VI) CHAIN PRECURSOR. ;contains MER22.t1 MSR1 repetitive element ; mRNA sequence, reliable 3' end

Table. 10 Genes differentially expressed in endothelial cells from DCIS and normal breast tissue

SEQ ID NO:	Tag_Sequence	NL	D6	d6/n	Unigene	Gene
1809	ATGTTAGTGT	21	0	-8	71573	Hypothetical protein FLJ10074, internal tag
1810	GAAATCCAAA	23	1	-9	248396	EST, Moderately similar to C35863 tryptase (EC 3.4.21.59) III precursor - human, reliable 3' end
1811	GGGGGGGGGG	23	0	-9	329973	EST, Weakly similar to 0903209A peptide PD,basic Pro rich [Homo sapiens], reliable 3' end
1812	GACATCAAGT	23	0	-9	182265	keratin 19, reliable 3' end
1813	CTCGCGCTGG	23	0	-9	25640	claudin 3, reliable 3' end
1814	CCTGCCCACC d	26	1	-10	1892	phenylethanolamine N-methyltransferase, reliable 3' end
1815	CTCACCGCCC d	29	1	-11	183650	cellular retinoic acid binding protein 2, reliable 3' end
1816	AGGAGCGGGG d	29	1	-11	252189	Syndecan 4 (amphiglycan, ryudocan), undefined 3' end
1817	TCCCTATGAA d	29	0	-11		no match
1818	GGAACAAACA d	29	0	-11	286124	CD24 antigen (small cell lung carcinoma cluster 4 antigen), reliable 3' end
1819	TCCCTATGAA d	29	0	-11		no match
1820	TAGGTCCCCT d	29	0	-11	82985	Collagen, type V, alpha 2, internal tag
1821	TCCGTATTAA d	31	0	-12		no match
1822	TCCGTATTAA d	31	0	-12		no match
1823	GGCTGCCCAG d	34	1	-13	172210	MUF1 protein, reliable 3' end
1824	TTCGGTTGGT d	34	0	-13	BG939135	cn30g02.x1 Normal Human Trabecular Bone Cells Homo sapiens cDNA clone NHTBC_cn30g02 random, mRNA sequence, undefined 3' end
1825	TCCCTAGTAA d	36	0	-14		no match
1826	AGCTGTCCCC d	39	1	-15	X93334	mitochondrial
1827	ACCTGCACAA d	39	0	-15	BM690922	UI-E-CI1-aaz-e-11-0-UI.r1 UI-E-CI1 Homo sapiens cDNA clone UI-E-CI1-aaz-e-11-0-UI 5', mRNA, undefined 3' end
1828	CCGGGGGAGC d	44	1	-17	172928	collagen, type I, alpha 1, internal tag
1829	GCCTACCCGA d	49	1	-19	23582	tumor-associated calcium signal transducer 2, reliable 3' end
1830	TCCCTATTAA d	2798	43	-35		no match
1831	ATCGTGGCGG d	177	0	-68	5372	Claudin 4, reliable 3' end

Table 11. Genes from Table 7 encoding secreted and cell surface proteins

Unigene	Gene
375570	HLA-DRB1, major histocompatibility complex, class II, DR beta 1
126256	interleukin 1, beta
76807	major histocompatibility complex, class II, DR alpha
73817	small inducible cytokine A3
169401	apolipoprotein E
79356	Lysosomal-associated multispanning membrane protein-5, haematopoetic cell specific
179657	plasminogen activator, urokinase receptor
17409	cysteine-rich protein 1 (intestinal)
74631	basigin (OK blood group), leukocyte activation M6 antigen
814	major histocompatibility complex, class II, DP beta 1
352107	trefoil factor 3 (intestinal)

Table 12. Genes from Table 8 encoding secreted or cell surface proteins

Unigene	Gene
119571	Collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant, shorter alternative transcript
172928	collagen, type I, alpha 1, internally primed site
102171	immunoglobulin superfamily containing leucine-rich repeat, reliable 3' end
128087	F2R coagulation factor II (thrombin) receptor, reliable 3' end
172928	collagen, type I, alpha 1, internal tag
108623	thrombospondin 2, reliable 3' end
278568	H factor (complement)-like 1, reliable 3' end
159263	collagen, type VI, alpha 2, reliable 3' end
265827	G1P3 interferon alpha-inducible protein, reliable 3'end, 97%, IFI-6-16, secreted based on PSORT
296049	microfibrillar-associated protein, undefined 3' end
274313	insulin-like growth factor binding protein 6, reliable 3' end
75736	apolipoprotein D, reliable 3' end
36131	collagen, type XIV, alpha 1 (undulin), reliable 3' end
11590	cathepsin F, reliable 3' end
24395	small inducible cytokine subfamily B (Cys-X-Cys), member 14 (BRAK), reliable 3' end
76152	decorin, reliable 3' end
89137	Low density lipoprotein-related protein 1 (alpha-2-macroglobulin receptor), reliable 3' end
289019	latent transforming growth factor beta binding protein 3, relable 3' end
2420	superoxide dismutase 3, extracellular, reliable 3' end
172928	collagen, type I, alpha 1, shorter alternative transcript
245188	tissue inhibitor of metalloproteinase 3 (Sorsby fundus dystrophy, pseudoinflammatory), shorter alternative transcript
821	biglycan, reliable 3' end
75736	apolipoprotein D, internal tag
172928	collagen, type I, alpha 1, internal tag
76294	CD63 antigen (melanoma 1 antigen) reliable 3' end
172928	collagen, type I, alpha 1, internal tag
79732	fubulin, transcript variant C, reliable 3' end
1279	C1R Complement component 1, r subcomponent, reliable 3' end
277477	HLA-C Major histocompatibility complex, class I, C, reliable 3' end

Table 12. Genes from Table 8 encoding secreted or cell surface proteins

Unigene	Gene
283713	collagen triple helix repeat containing 1, reliable 3' end
193716	Complement component (3b/4b) receptor 1, including Knops blood group system, reliable 3' end
155597	DF D component of complement (adipsin), internal tag
54457	CD81 antigen (target of antiproliferative antibody 1), reliable 3' end
93913	interleukin 6 (interferon, beta 2), reliable 3' end
101382	tumor necrosis factor, alpha-induced protein 2, reliable 3' end
29352	tumor necrosis factor, alpha-induced protein 6, internally primed site
119206	insulin-like growth factor binding protein 7, reliable 3' end
78056	cathepsin L, reliable 3' end
202097	procollagen C-endopeptidase enhancer, reliable 3' end
237356	stromal cell-derived factor 1, SAGE Genie: no match, NCBI: Acc.no.U19495
83942	cathepsin K (pycnodysostosis), reliable 3' end
177543	MIC2 antigen identified by monoclonal antibodies 12E7, F21 and O13, reliable 3' end, Tcells?
170040	platelet-derived growth factor receptor-like, reliable 3' end
151242	serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1, (angioedema, hereditary), reliable 3' end
149609	integrin, alpha 5 (fibronectin receptor, alpha polypeptide), reliable 3'end
135084	cystatin C (amyloid angiopathy and cerebral hemorrhage), reliable 3' end
75111	protease, serine, 11 (IGF binding), reliable 3' end
111334	FTL Ferritin, light polypeptide, reliabe 3' end
24395	small inducible cytokine subfamily B (Cys-X-Cys), member 14 (BRAK), reliable 3' end
108885	collagen, type VI, alpha 1, reliable 3' end
169401	apolipoprotein E, undefined 3' end
227751	lectin, galactoside-binding, soluble, 1 (galectin 1), reliable 3' end
296267	follistatin-like 1, reliable 3' end
119178	Cation-chloride cotransporter-interacting protein, reliable 3' end
136348	Osteoblast specific factor 2 (fasciclin I-like), undefined 3' end
111301	Matrix metalloproteinase 2 (gelatinase A, 72kD gelatinase, 72kD type IV collagenase, reliable 3' end
75415	beta-2-microglobulin, reliable 3' end

Table 12. Genes from Table 8 encoding secreted or cell surface proteins

Unigene	Gene
62954	Ferritin, heavy polypeptide 1, reliable 3' end
287797	integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12), reliable 3' end
74471	Gap junction protein, alpha 1, 43kD (connexin 43), reliable 3' end
8867	cysteine-rich, angiogenic inducer, 61, reliable 3' end
87409	thrombospondin 1, reliable 3' end
23582	tumor-associated calcium signal transducer 2, reliable 3' end
624	interleukin 8, reliable 3' end
82689	tumor rejection antigen (gp96) 1, reliable 3' end
1369	Decay accelerating factor for complement (CD55, Cromer blood group system), reliable 3' end
171921	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C, reliable 3' end
303649	small inducible cytokine A2 (monocyte chemotactic protein 1), reliable 3' end
77356	transferrin receptor (p90, CD71), reliable 3' end
9006	VAMP (vesicle-associated membrane protein)-associated protein A (33kD), reliable 3' end
6418	seven transmembrane domain orphan receptor, reliable 3' end
78614	complement component 1, q subcomponent binding protein, reliable 3' end
287797	ITGB1 Integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12), internally primed site
75765	GRO2 oncogene, reliable 3' end
78225	annexin A1, reliable 3' end
2820	oxytocin receptor, reliable 3' end
117938	Collagen, type XVII, alpha 1, reliable 3' end
289114	hexabrachion (tenascin C, cytotactin), reliable 3' end
799	diphtheria toxin receptor (heparin-binding epidermal growth factor-like growth factor), reliable 3' end
2250	leukemia inhibitory factor (cholinergic differentiation factor), reliable 3' end
198689	bullous pemphigoid antigen 1 (230/240kD), reliable 3' end
8230	a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 1, reliable 3' end

Table 13. Genes from Table 9 encoding secreted or cell surface proteins

Unigene	Gene
277477	HLA-C Major histocompatibility complex, class I, C, reliable 3' end
332053	serum amyloid A1, reliable 3' end
164021	Small inducible cytokine subfamily B (Cys-X-Cys), member 6 (granulocyte chemotactic protein 2), reliable 3' end
297681	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1, reliable 3' end
69771	B-factor, properdin, reliable 3' end, complement factor
350470	Trefoil factor 1 (breast cancer, estrogen-inducible sequence expressed in), reliable 3' end
112341	protease inhibitor 3, skin-derived (SKALP), reliable 3' end
75498	small inducible cytokine subfamily A (Cys-Cys), member 20, reliable 3' end
2250	leukemia inhibitory factor (cholinergic differentiation factor), internal tag
155223	stanniocalcin 2, reliable 3' end
54457	CD81 antigen (target of antiproliferative antibody 1), reliable 3' end
234726	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3, reliable 3' end
62492	HIN-1, secretoglobin, family 3A, member 1, reliable 3' end
89690	GRO3 oncogene, reliable 3' end
204096	secretoglobin, family 1D, member 2, reliable 3' end
278573	CD59 antigen p18-20 (antigen identified by monoclonal antibodies 16.3A5, EJ16, EJ30, EL32 and G344), reliable 3'end, similarity to urokinase plasminogen activator receptor
621	lectin, galactoside-binding, soluble, 3 (galectin 3), reliable 3' end
789	GRO1 oncogene (melanoma growth stimulating activity, alpha), reliable 3' end
93913	interleukin 6 (interferon, beta 2), reliable 3' end
348419	LOC118430 Small breast epithelial mucin, undefined 3' end
75106	clusterin (complement lysis inhibitor, SP-40,40, sulfated glycoprotein 2, testosterone-repressed prostate message 2, apolipoprotein J), reliable 3' end
277477	HLA-C Major histocompatibility complex, class I, C, reliable 3'end, 97%
75765	GRO2 oncogene, reliable 3' end
624	interleukin 8, reliable 3' end
119178	Cation-chloride cotransporter-interacting protein, reliable 3' end
5372	claudin 4, reliable 3' end
306226	Transmembrane gamma-carboxyglutamic acid protein 4, reliable 3' end
31439	serine protease inhibitor, Kunitz type, 2, reliable 3' end

Table 13. Genes from Table 9 encoding secreted or cell surface proteins	
Unigene	Gene
323910	V-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian), undefined 3' end

Table 14. Genes from Table 10 encoding secreted or cell surface proteins

Unigene	Gene
119206	insulin-like growth factor binding protein 7, shorter alternative transcript
16085	putative G-protein coupled receptor, reliable 3' end
25590	stanniocalcin 1, reliable 3' end
74561	alpha-2-macroglobulin, reliable 3' end
1516	insulin-like growth factor binding protein 4, undefined 3' end
352392	major histocompatibility complex, class II, DR beta 5
119129	collagen, type IV, alpha 1, reliable 3' end
79368	epithelial membrane protein 1, reliable 3' end
211604	a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 4, reliable 3' end
119206	insulin-like growth factor binding protein 7, reliable 3' end
1908	proteoglycan 1, secretory granule, reliable 3' end
74471	Gap junction protein, alpha 1, 43kD (connexin 43), reliable 3' end
624	interleukin 8, reliable 3' end
89546	selectin E (endothelial adhesion molecule 1), reliable 3' end
168383	intercellular adhesion molecule 1 (CD54), human rhinovirus receptor, reliable 3 'end
298275	solute carrier family 38, member 2, reliable 3' end
78409	collagen, type XVIII, alpha 1, shorter alternative transcript
277477	Major histocompatibility complex, class I, C, reliable 3' end
75445	SPARC-like 1 (mast9, hevin), reliable 3' end
111334	Ferritin, light polypeptide, reliable 3' end
351316	Transmembrane 4 superfamily member 1, reliable 3' end
111779	secreted protein, acidic, cysteine-rich (osteonectin), reliable 3' end
75415	beta-2-microglobulin, reliable 3' end
181357	laminin receptor 1 (67kD, ribosomal protein SA), reliable 3' end
172928	collagen, type I, alpha 1, internally primed site
300697	immunoglobulin heavy constant gamma 3 (G3m marker), reliable 3' end
119571	Collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant), shorter alternative transcript
75111	protease, serine, 11 (IGF binding), similar to IGFBP7, cleaves IGF
75511	connective tissue growth factor, undefined 3'end, 79.6%
193716	Complement component (3b/4b) receptor 1, including Knops blood group system, reliable 3' end
172928	Collagen, type I, alpha 1, internal tag
93557	proenkephalin (NCBI only)
158287	syndecan 3 (N-syndecan)
89137	Low density lipoprotein-related protein 1 (alpha-2-macroglobulin receptor), reliable 3' end
83326	matrix metalloproteinase 3 (stromelysin 1, progelatinase), reliable 3' end
108623	Thrombospondin 2, reliable 3' end
102171	immunoglobulin superfamily containing leucine-rich repeat, reliable 3' end
25640	claudin 3, reliable 3' end
252189	Syndecan 4 (amphiglycan, ryudocan), undefined 3' end
286124	CD24 antigen (small cell lung carcinoma cluster 4 antigen), reliable 3' end
BG939135	cn30g02.x1 Normal Human Trabecular Bone Cells Homo sapiens cDNA clone NHTBC_cn30g02 random, mRNA sequence, undefined 3' end
172928	collagen, type I, alpha 1, internal tag
23582	tumor-associated calcium signal transducer 2, reliable 3' end
5372	Claudin 4, reliable 3' end

Example 7. Analysis of SAGE libraries from epithelial cells and non-epithelial cells of normal breast tissue and breast tissues from patients with various diseases of the breast

SAGE analyses were performed on cell types in addition to those described in Example 6 and on breast tissue from patients with a variety of breast conditions. The data described in Example 6 and additional data were analyzed in a manner different to that described in Example 6.

To determine the molecular profile of various cell types that are found in normal and diseased breast tissue (e.g., cancerous epithelial and non-cancerous stromal cells within a breast tumor) and to identify autocrine and paracrine interactions that may play a role in breast tumor progression, a purification procedure (similar to that described in Example 1 for the analysis described in Example 6) was developed that allows the isolation of pure cell populations from normal breast tissue, in situ (DCIS; ductal carcinoma in situ) and invasive breast carcinomas (Fig. 5A). Cell type-specific surface markers and magnetic beads were used for the rapid sequential isolation of the various cell types. The BerEP4 antigen that is restricted to epithelial cells, the CD45 pan-leukocyte marker, and the P1H12 antibody that specifically recognizes endothelial cells were exploited for this purpose. The CD10 antigen is present in myoepithelial cells and myofibroblasts but also in some leukocytes. Thus, to minimize the cross contamination of these different cell types, in the case of normal and DCIS breast tissue, myoepithelial cells were isolated from organoids (breast ducts). On the other hand, in invasive tumors, leukocytes were removed prior to capturing the myofibroblasts using the CD10 beads. There is no antibody is available that specifically recognizes fibroblasts and thereby facilitates their purification. Thus, the unbound fraction, following removal of all other cell types, was used as a fibroblast-enriched "stroma" fraction.

This cell purification protocol includes enzymatic digestion of the tissue and the possibility that the expression of some genes could be altered due to the procedure cannot be excluded. However, in that it was possible to verify the SAGE data by alternative methods using unprocessed tissue (see below), any such hypothetical changes are likely to be minimal. The success of the purification method and the purity of each cell fraction were confirmed by performing RT-PCR on a small fraction of the isolated cells using cell type-specific genes as was done for the cell fractions described in Example 6 (see Example 1). The remaining portion of the

cells (~10,000-100,000 cells depending on the sample) was used for the generation of micro-SAGE libraries following previously described protocols and for the isolation of genomic DNA to be used for array-Comparative Genomic Hybridization (aCGH) and Single Nucleotide Polymorphism (SNP) array studies [Porter et al. (2003a) Mol. Cancer Res. 1:362-375; Porter et al. (2001)].

SAGE libraries were generated using a modified micro-SAGE protocol and the I-SAGE or long I-SAGE kits from Invitrogen (Carlsbad, CA). Approximately 50,000 tags (mean average tag number 56,647±4,383) were obtained from each library, and the preliminary analysis of the SAGE data was performed essentially as described [Porter et al. (2001)]. Briefly, genes significantly ($p \leq 0.002$) differentially expressed between normal and cancerous cells were identified by performing pair-wise comparisons using the SAGE2000 software that includes the software to perform Monte Carlo analysis (obtained from Johns Hopkins University, Baltimore, MD).

SAGE libraries were generated from epithelial cells, and myoepithelial cells (and myofibroblasts from invasive tumors), infiltrating leukocytes, endothelial cells, and fibroblasts ("stroma") from one normal breast reduction tissue, two different DCIS, and three invasive breast tumors. Not all libraries were generated from all cases due to the inability to obtain sufficient amounts of purified cells. In addition, a fibroadenoma and a phyllodes tumor were included in the SAGE analysis. Fibroadenomas are the most common benign breast tumors and are not considered to progress to malignancy despite genetic changes detected in the stromal (but not epithelial) cells [Amiel et al. (2003) Cancer Genet. Cytogenet. 142:145-148]. Phyllodes tumors, on the other hand, are rare fibroepithelial tumors that are usually benign but can recur and progress to malignant sarcomas. Phyllodes tumors were initially considered stromal neoplasms but recent molecular studies demonstrating frequently discordant genetic alterations in both epithelial and stromal cells suggest that phyllodes tumors may represent a true clonal co-evolution of malignant epithelial and stromal cells [Sawyer et al. (2000) Am. J. Pathol. 156:1093-1098; Sawyer et al. (2002) J. Pathol. 196: 437-444]. Analysis of the SAGE data confirmed that the cell purification procedure worked well in that several genes known to be specific for a particular cell type were present in the appropriate SAGE libraries. For example cytokeratins 8 and 19, E-cadherin, HIN-1, CD24 were highly specific for epithelial cells, myofibroblast and myoepithelial cells demonstrated high levels of smooth muscle actin, various

extracellular matrix proteins including collagens, and matrix metalloproteinases, while leukocyte libraries had the highest levels of several chemokines and lysozyme.

Based on statistical methods developed (by bioinformaticians in the Department of Research Computing at the Dana-Farber Cancer Institute and the Department of Biostatistics at the Harvard School of Public Health) for the analysis of SAGE data, genes that are specifically expressed in a particular cell type and tumor progression stage were identified. Genes were defined as specific for a particular cell type if the average tag number in all the SAGE libraries generated from the selected cell type was statistically significantly ($P<0.02$) different from that of all other cell types. Using these criteria, 357 tags were identified as discriminating epithelial cells from other cell types, 572 tags were identified as discriminating myoepithelial cells and myofibroblasts from all other cell types, 502 tags were identified as discriminating leukocytes from all other cell types, 124 tags were identified as discriminating endothelial cells from all other cell types, and 604 tags were identified as discriminating "stromal" cells depleted of all the above-listed cell types (i.e., mostly fibroblasts) from all other cell types.

To further define SAGE tags specific for each cell type, within each group of tags, those that were not only statistically significantly different, but also more abundant in the specific cell type, were selected. This led to the identification of 70 tags that were most abundant in epithelial cells, 117 tags present at highest levels in myoepithelial cells and myofibroblasts, 70 tags highly expressed in leukocytes, 117 tags in stroma, and 78 endothelium-specific tags. Several of these genes have previously been described as being specific for a particular cell type, e.g., keratins 8 and 19 for epithelial cells, keratins 14 and 17 for myoepithelial cells, and chemokines and chemokine receptors for leukocytes [Page et al. (1999) Proc. Natl. Acad. Sci. USA 96:12589-12594]. However, the cell type-specific expression of the majority of the genes has not been previously documented. The majority of the transcripts corresponding to these cell-type specific SAGE tags encode known genes but a significant fraction either are uncharacterized ESTs or currently have no cDNA match (~10% of the tags on average belong to each of these latter groups). In stroma 25/117 tags (21%) had no database match suggesting that they correspond to previously unidentified transcripts.

Next, using the 471 SAGE tags most abundantly expressed or 63 of the SAGE tags most highly specifically present in each of the five cell types, a clustering analysis of all 27 SAGE libraries using a new Poisson model based K-means algorithm (PK algorithm) was performed in

order to delineate similarities and differences among the samples. In addition, a clustering analysis of the SAGE libraries using each of the cell type specific genes was performed. The PK clustering method orders the samples according to their relatedness. For example, using the 63 most highly cell type specific SAGE tags, a division of the 27 SAGE libraries according to cell types was obtained and, within each cell type sub-group, the DCIS samples are located between normal breast tissue and invasive breast cancer SAGE libraries. These results confirmed that, not only tumor epithelial cells, but also other cell types in the tumor are different from their corresponding normal counterparts. Since these differences are already pronounced at a pre-invasive (DCIS) tumor stage, they suggest a role for stromal changes not only in tumor invasion and metastasis, but also in the earlier steps of breast tumorigenesis.

The most consistent and dramatic gene expression changes were found to occur in myoepithelial cells. Over 300 genes were differentially expressed at $p<0.002$ in both DCIS myoepithelial libraries. Interestingly, a significant fraction (89 out of 245 known genes) of these genes encode secreted or cell surface proteins, suggesting extensive abnormal paracrine interactions between myoepithelial and other cell types. Myoepithelial cells are thought to be derived from bi-potential stem cells that also give rise to luminal epithelial cells, although recently another progenitor has also been identified that can differentiate only to myoepithelial cells [Bocker et al. (2002) Lab. Invest. 82:737-746; Dontue et al. (2003) Genes Dev. 17:1253-1270]. The function of myoepithelial cells and their role in breast cancer is not well understood. However, myoepithelial cells have been shown to be able to suppress breast cancer cell growth, invasion, and angiogenesis [Deugnier et al. (2002) Breast Cancer Res. 4:224-230; Sternlicht and Barsky (1997) Clin. Cancer Res. 3:1949-1958]. The main distinguishing feature between in situ and invasive carcinomas, which is also used as a diagnostic criterion, is that: (a) in DCIS the cancer epithelial cells are separated from the stroma by a nearly continuous layer of myoepithelial cells and basement membrane; while (b) in invasive and metastatic tumors cancer cells are admixed with stroma.

In Table 15 are shown the most highly cell type-specific SAGE tags and corresponding genes. Columns 1-27 in Table 15 show data obtained from 27 separate libraries generated from cells from a variety of samples. These samples were:

Columns 1-7 (myoepithelial cells and myofibroblasts):

Column 1: myoepithelial cells isolated from normal breast tissue adjacent to invasive ductal carcinoma (IDC7) tissue.

Column 2: myoepithelial cells isolated from reduction mammoplasty normal breast tissue (RM1).

Column 3: myofibroblasts isolated from an invasive ductal carcinoma (IDC7).

Column 4: myofibroblasts isolated from an invasive ductal carcinoma (IDC8).

Column 5: myofibroblasts isolated from an invasive ductal carcinoma (IDC9).

Column 6: myoepithelial cells isolated from DCIS tissue (D7).

Column 7: myoepithelial cells isolated from DCIS tissue (D6).

Columns 8-10 and 26 (fibroblast-enriched cells):

Column 8: fibroblast-enriched cells from an invasive ductal carcinoma (IDC7).

Column 9: fibroblast-enriched cells from DCIS tissue (D6).

Column10: fibroblast-enriched cells from reduction mammoplasty normal breast tissue (RM2).

Column 26: fibroblast-enriched cells from a phyllodes tumor.

Columns 11-12 (endothelial cells):

Column 11: endothelial cells isolated from reduction mammoplasty normal breast tissue (RM2).

Column 12: endothelial cells isolated from DCIS tissue (D6).

Columns 13-16 (leukocytes):

Column 13: leukocytes isolated from DCIS tissue (D7).

Column 14: leukocytes isolated from DCIS tissue (D6).

Column 15: leukocytes isolated from an invasive ductal carcinoma (IDC7).

Column 16: leukocytes isolated from reduction mammoplasty normal breast tissue (RM2).

Columns 17-25 (epithelial cells; luminal type):

Column 17: epithelial cells isolated from an invasive ductal carcinoma (IDC7).

Column 18: epithelial cells isolated from an invasive ductal carcinoma (IDC8).

Column 19: epithelial cells isolated from an invasive ductal carcinoma (IDC9).

Column 20: epithelial cells isolated from DCIS tissue (D7).

Column 21: epithelial cells isolated from DCIS tissue (D6).

Column 22: epithelial cells isolated from normal breast tissue adjacent to DCIS (D2) tissue.

Column 23: epithelial cells isolated from reduction mammoplasty normal breast tissue (RM3).

Column 24: epithelial cells isolated from DCIS tissue (D2).

Column 25: epithelial cells isolated from DCIS tissue (D3).

Column 27: (unseparated cells of a juvenile fibroadenoma)

Rows 1-72 in Table 15 show SAG tags detected in the various libraries depicted in columns 1-27.

Rows 1-27: SAGE tags that were statistically significantly ($p < 0.02$) more abundantly expressed in epithelial cells than in all other cell types.

Rows 28-53: SAGE tags that were statistically significantly ($p < 0.02$) more abundantly expressed in myoepithelial cells than in all other cell types or in myofibroblasts than in all other cell types.

Rows 54-58: SAGE tags that were statistically significantly ($p < 0.02$) more abundantly expressed in leukocytes than in all other cell types.

Rows 59-65: SAGE tags that were statistically significantly ($p < 0.02$) more abundantly expressed in fibroblast-enriched cells than in all other cell types.

Rows 66-72: SAGE tags that were statistically significantly ($p < 0.02$) more abundantly expressed in endothelial cells than in all other cell types.

From Table 15 it can readily be determined, by referring to the intersection of relevant columns and rows, which of the listed genes are differently expressed (more highly or at a lower level) in the various cell types from DCIS and/or invasive breast cancers compared to corresponding cell types from normal tissue. Analogous differences in expression between cells from DCIS and from invasive breast carcinomas can similarly be discerned from the data in Table 15. It is noted that myofibroblasts are cells found only in cancer tissue and thus comparisons of gene expression involving myofibroblasts will be between: (a) myofibroblasts in DCIS and invasive breast carcinomas; or (b) between myofibroblasts in DCIS or invasive breast carcinomas and any other cell type (e.g., myoepithelial cells or fibroblasts) from normal breast tissue.

Follow up studies were focused on myoepithelial cells, with special emphasis on secreted proteins and receptors abnormally expressed in these cells. Several proteases [e.g., cathepsins F, K, and L, MMP2 (matrix metalloproteinase 2), and PRSS11 (protease serine (insulin-like growth factor-binding)], protease inhibitors [thrombospondin 2, SERPING1 (serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor) member 1), cystatin C, and TIMP3 (tissue inhibitor

of metalloproteinase 3)], and many different collagens were highly up-regulated in DCIS myoepithelial cells, suggesting a role for these cells in extracellular matrix remodeling (Table 16).

In Table 16, the column labeled "N-MYOEP-1" shows data obtained from a SAGE library generated from myoepithelial cells isolated from reduction mammoplasty normal breast tissue (RM1). The columns labeled "D-MYOEP-7" and "D-MYOEP-6" show data obtained from a SAGE library generated from myoepithelial cells isolated from two DCIS tissue samples (D7 and D6, respectively). The column labeled "Ratio D/N" shows the ratio of the average of the numbers of SAGE tags obtained with the two DCIS tissue samples to the SAGE tag number obtained with normal breast tissue.

Array-Comparative Genomic Hybridization (aCGH) and Single Nucleotide Polymorphism (SNP) array studies indicated that the changes in gene expression in non-cancer cells present in breast tumor tissue detected by the analysis described in Example 6 and this Example were not due to chromosomal gains or losses, e.g., loss of heterozygosity.

Table 15. List of most highly cell type-specific SAGE tags and corresponding genes

	SAGE tag	SEQ ID NO:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	Unigene	Gene description
1	CCTCCAGCTA	1832	0	5	9	6	0	0	2	28	0	10	8	0	2	4	31	11	118	72	124	159	32	28	62	43	14	3	25	356123	KRT8 keratin 8
2	GACATCAAGT	1833	0	0	5	0	0	0	0	15	0	4	9	0	5	9	26	11	73	64	59	153	48	15	18	55	2	0	5	309517	KRT19 keratin 19
3	TGTGGGTGCT	1834	0	5	2	0	0	0	3	3	0	2	0	0	0	0	4	0	11	17	25	49	83	14	15	14	5	0	5	194657	CDH1 cadherin 1, type 1, E-cadherin
4	AGGAAGGAAC	1835	0	0	0	2	0	0	0	2	0	0	0	0	0	3	0	0	18	0	2	24	90	0	0	3	7	0	3	446352	ERBB2
5	CTGGCCCTCG	1836	0	0	0	9	0	0	0	2	0	0	4	0	0	2	3	4	33	149	74	74	10	62	163	39	6	0	5	350470	TFF1 trefoil factor 1
6	CTCCACCCGA	1837	2	0	3	19	0	0	0	5	0	4	8	0	0	8	12	38	43	297	51	38	25	3	19	284	11	3	50	82961	TFF3 trefoil factor 3
7	AAGCTCGCCG	1838	0	0	2	2	0	0	0	0	0	3	2	0	0	3	0	7	0	24	0	0	7	19	69	0	0	0	0	62492	SCGB3A1 secretoglobin, family 3A, member 1 (HIN-1)
8	CTTCCTGTGA	1839	0	0	0	0	0	0	0	0	0	5	0	0	0	6	0	0	2	5	0	5	67	98	272	7	10	0	0	348419	LOC118430 small breast epithelial mucin
9	AAGAAAACCT	1840	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	22	10	19	22	2	2	8	16	0	0	3	100686	BCMP11 breast cancer membrane protein 11
10	ATTTTCTAAA	1841	0	0	0	8	0	0	0	2	0	0	0	0	0	0	3	0	8	68	13	5	6	3	2	25	0	0	3	226391	AGR2 anterior gradient 2 homolog (Xenopus laevis)
11	CGGACTCACT	1842	0	2	3	2	2	0	0	0	0	2	4	3	2	0	0	0	9	23	13	89	12	0	3	11	3	0	3	300446	STARD10 START domain containing 10
12	GGAACAAACA	1843	0	0	3	0	0	0	0	11	0	8	11	0	6	6	9	14	62	7	129	94	122	62	30	57	3	0	13	375108	CD24 CD24 antigen
13	AATATGTGGG	1844	13	9	7	17	9	2	0	29	6	6	3	6	0	0	14	0	89	56	80	112	2	2	6	235	4	8	12	98664	BPA-1 mRNA for brain peptide A1
14	GGACTCTGGA	1845	0	0	4	0	0	0	0	2	0	6	3	0	0	5	7	5	25	39	2	23	31	14	56	11	7	0	0	439027	BDNF brain-derived neurotrophic factor
15	CTGGCCCTCG	1846	0	0	0	9	0	0	0	2	0	0	4	0	0	2	3	4	33	149	74	74	10	62	163	39	6	0	5	43654	CLN6 ceroid-lipofuscinosis, neuronal 6, late infantile,
16	ATCGTGGCGG	1847	0	0	60	2	0	7	0	61	0	7	68	0	19	11	69	27	357	36	96	972	86	57	23	36	20	0	0	5372	CLDN4 claudin 4
17	ATCGTGGCGG	1848	0	0	60	2	0	7	0	61	0	7	68	0	19	11	69	27	357	36	96	972	86	57	23	36	20	0	0	8026	SESN2 sestrin 2
18	GCAGGGCCTC	1849	0	0	9	5	0	0	6	4	0	8	4	0	2	3	0	9	29	39	15	68	16	21	19	44	8	0	15	301350	FXYD3 FXYD domain containing ion transport
19	TGTGGGTGCT	1850	0	5	2	0	0	0	3	3	0	2	0	0	0	0	4	0	11	17	25	49	83	14	15	14	5	0	5	306339	SRPUL sushi-repeat protein
20	GGACTCTGGA	1851	0	0	4	0	0	0	0	2	0	6	3	0	0	5	7	5	25	39	2	23	31	14	56	11	7	0	0	512643	AZGP1 alpha-2-glycoprotein 1, zinc
21	ATGCTCAGCC	1852	0	0	4	2	0	0	0	3	0	0	0	0	0	0	3	0	9	12	86	48	9	2	4	6	0	0	0	96125	RCP Rab coupling protein
22	AAATAAAGAA	1853	2	2	3	5	0	0	0	10	2	3	2	0	0	2	9	0	33	22	61	28	19	7	5	13	2	0	9	389700	MGST1 microsomal glutathione S-transferase 1
23	GCAGTGGCCT	1854	2	0	0	0	0	0	0	6	0	3	5	0	0	4	0	2	8	26	14	25	11	3	3	32	5	0	11	396783	SLC9A3R1 solute carrier family 9, isoform 3 regulatory
24	TGGGGTTCTT	1855	0	0	0	0	0	0	0	0	0	0	4	0	2	0	0	0	8	5	0	84	0	0	0	0	0	0	0	272499	DHRS2 dehydrogenase/reductase (SDR family)
25	ATGCTCAGCC	1856	0	0	4	2	0	0	0	3	0	0	0	0	0	0	3	0	9	12	86	48	9	2	4	6	0	0	0	98306	KIAA1862 KIAA1862 protein
26	TTGCGTTGCG	1857	0	0	0	0	0	0	0	0	0	0	4	0	0	2	0	3	0	0	0	4	16	0	0	0	89	0	0	no match	
27	TCTCCATACC	1858	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	137	0	183	0	0	0	no match	
28	GATGTGCACG	1859	0	339	41	5	0	5	19	2	0	0	0	0	0	3	0	0	0	0	0	0	0	6	4	0	6	0	2	355214	KRT14 keratin 14
29	GACCAGCAGA	1860	0	0	40	15	24	18	44	0	0	3	13	3	4	6	0	0	0	0	0	0	0	0	0	0	0	0	4	137569	TP73L tumor protein p73-like
30	TTAAATAGCA	1861	6	0	57	80	181	2	14	11	2	6	8	0	0	0	3	0	2	10	4	0	0	0	0	0	0	18	19	172928	COL1A1 collagen, type I, alpha 1
31	CCGGGGGAGC	1862	3	0	43	52	104	46	55	8	0	7	17	0	0	4	0	0	0	6	2	2	2	0	0	0	0	18	10	172928	COL1A1 collagen, type I, alpha 1
32	GACTTTGGAA	1863	8	0	18	33	53	15	100	21	6	7	0	2	0	2	0	0	0	2	0	0	2	0	0	0	0	18	27	172928	COL1A1 collagen, type I, alpha 1
33	TGGAAATGAA	1864	4	0	11	16	18	3	24	4	0	6	0	2	0	0	2	0	0	0	0	0	0	0	0	0	0	5	15	172928	COL1A1 collagen, type I, alpha 1
34	CGGGGTGGCC	1865	8	0	22	11	9	81	22	5	0	2	0	0	4	2	2	0	0	3	0	3	4	0	0	0	0	3	0	1584	COMP cartilage oligomeric matrix protein
35	TGGAAGCAGA	1866	0	0	42	9	0	28	0	4	0	0	2	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	1584	COMP cartilage oligomeric matrix protein
36	CTGTCAGCGT	1867	5	0	70	34	107	12	29	5	2	3	0	3	0	3	6	0	0	2	3	0	4	0	0	0	0	9	0	283713	CTHRC1 collagen triple helix repeat containing 1
37	CAGGAGACCC	1868	0	0	33	51	302	0	8	8	2	0	0	0	0	3	2	0	2	3	4	0	0	0	0	0	0	0	0	143751	MMP11 matrix metalloproteinase 11 (stromelysin 3)
38	TCCCTACCGA	1869	0	0	10	15	22	0	4	0	2	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	367877	MMP2 matrix metalloproteinase 2
39	TGGAAGCAGA	1870	0	0	42	9	0	28	0	4	0	0	2	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	415041	THBS4 thrombospondin 4
40	AGAATGAGAT	1871	8	2	28	17	24	13	12	3	2	8	2	0	0	2	0	0	0	0	2	0	0	0	0	0	0	3	0	156316	DCN decorin
41	TATTTTCACA	1872	3	0	21	19	31	4	5	2	3	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	156316	DCN decorin
42	ACATAGACCG	1873	10	0	27	24	34	6	11	4	9	2	2	4	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	173594	SERPINF1
43	CTATAGGAGA	1874	4	2	13	19	61	2	4	9	5	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	13	7	274520	ANTXR1 anthrax toxin receptor 1
44	GTAAATATGG	1875	0	81	12	4	0	0	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	443518	BPAG1 bullous pemphigoid antigen 1, 230/240kDa
45	TTTGTGGGCA	1876	2	0	8	17	11	11	7	0	0	2	0	3	0	0	0	0	0	0	2	0	0	0	0	0	0	3	4	439184	RCN3 reticulocalbin 3, EF-hand calcium binding
46	GGGAAGGGAC	1877	0	52	6	0	0	0	2	3	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13144	ORMDL2 ORM1-like 2
47	GGGAAGGGAC	1878	0	52	6	0	0	0	2	3	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	431156	PPP2R1B protein phosphatase 2, regulatory subunit
48	CTTCCTTGCC	1879	8	785	179	34	4	7	63	19	7	27	0	2	0	3	8	0	6	0	17	6	3	5	15	2	33	0	4	449630	HBA2 hemoglobin, alpha 2
49	GGGGAAATCG	1880	96	22	57	103	112	228	177	19	59	75	71	120	30	330	32	34	59	88	149	188	151	41	90	38	22	45	96	446574	TMSB10 thymosin, beta 10
50	TATTTTCACA	1881	3	0	21	19	31	4	5	2	3	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	132131	Transcribed sequences
51	CCACGGGATT	1882	20	0	62	164	78	23	168	17	10	0	19	16	2	14	0	4	0	8	5	0	2	0	0	0	2	0	68	no match	
52	GGTCTTCAAG	1883	0	0	5	23	27	0	7	2	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	no match	
53	GTGCGCCGGA	1884	0	40	7	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	no match	
54	GAGCTGGAAA	1885	0	0	0	0	0	0	0	0	2	0	0	0	0	33	0	0	0	0	0	0	0	0	0	0	0	0	0	73875	FAH fumarylacetoacetate hydrolase
55	GAGCTGGAAA	1886	0	0	0	0	0	0	0	0	2	0	0	0	0	33	0	0	0	0	0	0	0	0	0	0	0	0	0	20950	LHPP phospholysine phosphohistidine inorganic

Table 15. List of most highly cell type-specific SAGE tags and corresponding genes

	SAGE tag	SEQ ID NO:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	Unigene	Gene description
56	GAGAAATCGT	1887	0	0	0	0	0	0	0	0	0	0	0	0	0	33	0	0	0	0	0	0	0	0	0	0	0	0	0	234734	LYZ lysozyme
57	AACGGGGCCC	1888	2	0	2	0	0	0	0	0	0	0	0	0	2	17	4	2	0	0	0	0	0	0	0	0	0	0	0	80420	CX3CL1 chemokine
58	ATTCCTGAGC	1889	2	0	0	0	0	0	0	0	0	0	0	0	2	24	0	2	0	0	0	0	0	0	0	0	0	0	0	no match	
59	ATACAGAATA	1890	2	0	0	0	0	2	0	0	0	64	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	169228	DLK1 delta-like 1 homolog
60	CAGGAGAAGG	1891	0	0	0	0	0	0	0	0	0	29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24049	GOLGA2 golgi autoantigen, golgin subfamily a, 2
61	CAGGAGAAGG	1892	0	0	0	0	0	0	0	0	0	29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	366	MGC27165 hypothetical protein MGC27165
62	GCGGAGGTGG	1893	2	0	0	0	0	2	0	2	0	283	4	11	10	6	2	4	0	0	0	0	0	0	0	0	0	0	0	366	MGC27165 hypothetical protein MGC27165
63	GCCGTTCTTA	1894	41	0	0	2	0	0	0	42	27	277	0	11	0	0	0	2	0	3	0	0	0	5	5	3	0	32	0	no match	
64	TGAACAGCAG	1895	2	0	0	0	0	0	0	4	5	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	no match	
65	GAGTTTATTC	1896	3	0	0	3	0	0	0	4	2	31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	no match	
66	AATGAATTAT	1897	0	0	0	0	0	0	0	0	0	0	3	9	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	293257	ECT2 epithelial cell transforming sequence 2 oncogene
67	TAGGTCAGGA	1898	0	0	0	0	0	0	0	0	0	0	7	4	0	2	0	0	0	0	0	0	0	0	0	0	0	0	2	43666	PTP4A3 protein tyrosine phosphatase type IVA,
68	CGAGAGTGTG	1899	0	0	0	0	0	0	0	0	0	0	4	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	175804	CDNA FLJ42395 fis, clone ASTRO2001076
69	GCGCCTCCCG	1900	0	0	0	0	0	0	0	0	0	0	11	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	435800	VIM vimentin
70	TGTTGAAAAA	1901	104	0	9	0	0	0	3	15	82	0	4	31	2	91	0	2	0	0	0	0	12	3	0	0	0	0	0	89546	SELE selectin E
71	AAGTTTGGTG	1902	0	0	0	0	0	0	0	0	0	0	3	12	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	66727	KCNJ10 potassium inwardly-rectifying channel,
72	GGCCGCGAGG	1903	0	0	0	0	0	3	0	2	0	0	18	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	78344	MYH11 myosin, heavy polypeptide 11, smooth muscle

Table 16. List of genes encoding secreted and cell surface proteins overexpressed in DCIS myoepithelial cells compared to normal myoepethelial cells

SEQ ID NO:	SAGE Tag	N-MYOEP-1	D-MYOEP-7	D-MYOEP-6	Ratio D/N	Unigene	Gene description
1904	ACCAAAAACC	2	274	849	244	172928	COL1A1 collagen, type I, alpha 1
1905	GATCAGGCCA	0	191	181	124	443625	COL3A1 collagen, type III, alpha 1
1906	TGGAAATGAC	0	50	228	93	172928	COL1A1 collagen, type I, alpha 1
1907	CGGGGTGGCC	0	193	24	73	1584	COMP cartilage oligomeric matrix protein
1908	CTAACGGGGC	0	169	20	63	513022	ISLR immunoglobulin superfamily containing leucine-rich repeat
1909	CAGATAAGTT	0	72	101	58	222171	KIAA0182 KIAA0182 protein
1910	CCGGGGGAGC	0	110	61	57	172928	COL1A1 collagen, type I, alpha 1
1911	GTCAAAATTT	0	110	47	52	458354	THBS2 thrombospondin 2
1912	GTGCTAAGCG	3	308	141	49	420269	COL6A2 collagen, type VI, alpha 2
1913	GACTTTGGAA	0	36	110	49	172928	COL1A1 collagen, type I, alpha 1
1914	CGCCGACGAT	0	100	32	44	287721	G1P3 interferon, alpha-inducible protein (clone IFI-6-16)
1915	TTGGGATGGG	0	103	29	44	296941	HFL1 H factor (complement)-like 1
1916	CATATCATTA	0	21	94	38	435795	IGFBP7 insulin-like growth factor binding protein 7
1917	TCCAGGAAAC	0	72	39	37	11590	CTSF cathepsin F
1918	GGCCCCTCAC	0	74	22	32	274313	IGFBP6 insulin-like growth factor binding protein 6
1919	ACATTCCAAG	0	50	42	31	245188	TIMP3 tissue inhibitor of metalloproteinase 3
1920	ATAAAAAGAA	0	19	73	31	83942	CTSK cathepsin K
1921	GACCAGCAGA	0	43	48	30	172928	COL1A1 collagen, type I, alpha 1
1922	ACTTATTATG	2	107	30	30	156316	DCN decorin
1923	GTGCGCTGAG	0	33	52	28	274485	HLA-C major histocompatibility complex, class I, C
1924	TGCGCTGGCC	0	67	18	28	289019	LTBP3 latent transforming growth factor beta binding protein 3
1925	AGGCTCCTGG	3	217	31	27	24395	CXCL14 chemokine
1926	CTCAACCCCC	2	105	19	27	162757	LRP1 low density lipoprotein-related protein 1
1927	CAGCGGCGGG	0	57	13	23	2420	SOD3 superoxide dismutase 3, extracellular
1928	GGCACCTCAG	2	36	65	22	512234	IL6 interleukin 6
1929	GCCTGTCCCT	0	50	13	21	821	BGN biglycan
1930	ATTTCTTCAA	0	19	44	21	31386	SFRP2 secreted frizzled-related protein 2
1931	TCGAAGAACC	2	60	34	21	445570	CD63 CD63 antigen
1932	ACATTCTTTT	0	17	44	20	389964	GPNMB glycoprotein (transmembrane)
1933	CTGTCAGCGT	0	29	32	20	283713	CTHRC1 collagen triple helix repeat containing 1
1934	CAGCTGGCCA	0	36	22	19	445240	FBLN1 fibulin 1
1935	ACTGAAAGAA	3	124	50	19	458355	C1S complement component 1, s subcomponent
1936	TTCTGTGCTG	3	105	40	16	376414	C1R complement component 1, r subcomponent
1937	GGATGTGAAA	0	19	26	15	283477	CD99 CD99 antigen
1938	ACTCAGCCCG	2	36	28	14	101382	TNFAIP2 tumor necrosis factor, alpha-induced protein 2
1939	TTTCCCTCAA	2	21	42	14	75111	PRSS11 protease, serine, 11 (IGF binding)
1940	CTAAAAAAAA	0	26	15	14	54457	CD81 CD81 antigen (target of antiproliferative antibody 1)
1941	GGCCACGTAG	0	26	15	14	155597	DF D component of complement
1942	AAGAAAGGAG	0	21	20	14	202097	PCOLCE procollagen C-endopeptidase enhancer
1943	GGAGGAATTC	0	21	20	14	418123	CTSL cathepsin L

Table 16. List of genes encoding secreted and cell surface proteins overexpressed in DCIS myoepithelial cells compared to normal myoepethelial cells

SEQ ID NO:	SAGE Tag	N-MYOEP-1	D-MYOEP-7	D-MYOEP-6	Ratio D/N	Unigene	Gene description
1944	AGCCACCGCG	2	43	19	14	355874	RABL2B RAB, member of RAS oncogene family-like 2B
1945	TGTAAACAAT	0	19	22	14	170040	PDGFRL platelet-derived growth factor receptor-like
1946	ACCTTGAAGT	2	36	19	12	407546	TNFAIP6 tumor necrosis factor, alpha-induced protein 6
1947	CATAAATGCG	0	21	13	12	436042	CXCL12 chemokine (stromal cell-derived factor 1)
1948	TTGCTGACTT	12	122	279	11	415997	COL6A1 collagen, type VI, alpha 1
1949	ATGGCAACAG	0	17	17	11	149609	ITGA5 integrin, alpha 5
1950	CTCTCCAAAC	2	26	20	10	384598	SERPING1 serine proteinase inhibitor, clade G, member 1
1951	TGCCTGCACC	5	76	46	9	304682	CST3 cystatin C
1952	GGAAATGTCA	18	93	325	8	367877	MMP2 matrix metalloproteinase 2
1953	CAGGTTTCAT	12	124	117	7	24395	CXCL14 chemokine
1954	CCGTGACTCT	12	112	70	5	433622	FSTL1 follistatin-like 1

Example 8. Evaluation of gene expression by immunohistochemistry and mRNA in situ hybridization

The generation of the SAGE libraries described in Example 7 involved initial *in vitro* cell purification steps that could potentially have altered *in vivo* gene expression patterns, although prior SAGE data from several laboratories suggest that these changes are likely to be minimal [Porter et al. (2003a); Porter et al. (2003b) Proc. Natl. Acad. Sci USA 100:10931-10936; St. Croix et al. (2000) Science 289:1197-1202]. Nevertheless, in order to further investigate the expression of selected genes at the cellular level *in vivo*, immunohistochemical and mRNA *in situ* hybridization analyses were performed on a panel of DCIS and invasive breast tumors (different from the tumors used for SAGE). In addition, the cell type specificity of some genes was verified by RT-PCR in the samples used for SAGE (data not shown).

Immunohistochemical analysis confirmed that two genes, those encoding IL-1β and CCL3 (MIP1α), are highly expressed in leukocytes infiltrating DCIS, but not normal breast tissue, whereas the CD45 (PTPRC) pan-leukocyte marker was expressed in both cases. Despite the similar number of total leukocytes in invasive tumors the frequency of IL-1β and CCL3 positive leukocytes, although higher than in normal breast tissue, was much lower than in DCIS, suggesting that in situ and invasive breast carcinomas may be immunologically dissimilar.

mRNA in situ hybridization determined that in DCIS tumors: (a) the expression of PDGF (platelet-derived growth factor) receptor β-like (PDGFRBL), cathepsin K (CTSK), and CXCL12 was localized to myofibroblasts as determined by smooth muscle actin (ACTA2) staining; (b) CXCL14 was expressed only in myoepithelial cells; (c) TIMP3, cystatin C (CST3) and collagen triple helix repeat containing 1 (CTHRC1) were expressed in both myoepithelial cells and myofibroblasts. In invasive tumors all these genes were expressed in myofibroblasts; there are no myoepithelial cells in invasive breast tumors. No signal was detected in normal breast tissue and with the sense probes (data not shown). Interestingly, although in DCIS tumors CXCL14 expression was detected only in myoepithelial cells, in some invasive breast carcinomas, while present in myofibroblasts, it was much more strongly expressed in tumor epithelial cells (data not shown). Similarly, some breast cancer cell lines expressed high levels of CXCL12 or CXCL14 *in vitro* suggesting that during tumor progression a paracrine factor may be converted into an autocrine one due to its up-regulation in the tumor epithelial cells. All the CXCL14 positive primary breast tumors and even the CXCL14 expressing breast cancer cell line

(UACC812) were obtained from young, pre-menopausal patients (average age of onset 39 years), suggesting a possible association of CXCL14 expression with clinico-pathologic characteristics of the tumors.

Example 9. The effect of CXCL12 and CXCL14 chemokines on breast cancer cells

The high level of expression of two chemokines, CXCL12 and CXCL14, in myoepithelial cells and myofibroblasts, both in DCIS and invasive breast carcinomas, was particularly interesting in view of the known function of chemokines as regulators of cell proliferation, differentiation, migration, and invasion [Gerard et al. (2001) Nat. Immunol. 2:108-115; Muller et al. (2001) Nature 410:50-56; Rossi et al. (2000) Annu. Rev. Immunol. 18:217-242]. To determine if CXCL12 and CXCL14 can act as autocrine and/or paracrine factors in breast tumors, an analysis to identify cell types expressing receptors for the two chemokines in primary breast tissue *in vivo* was carried out.

The signaling receptor for CXCL12 is CXCR4, which is known to be expressed in various lymphoid cells as well as a variety of epithelial cells [Gerard et al. (2001)]. The expression of CXCR4 in lymphoid and breast epithelial cells was confirmed by immunohistochemistry and SAGE data indicated that its expression is increased in invasive tumors compared to DCIS and normal breast tissue (data not shown).

The signaling receptor for CXCL14 is unknown but cell surface ligand binding experiments have suggested the presence of a putative CXCL14 receptor on monocytes and B-cells, suggesting that its receptor is unlikely to be CXCR4 [Kurth et al. (2001) J. Exp. Med. 194:855-861; Sleeman et al. (2000) Int. Immunol. 12:677-689]. To determine if a CXCL14-binding cell surface protein(s) is also present on breast cancer cells, an alkaline phosphatase-CXCL14 (AP-CXCL14) fusion protein to be used as a ligand in receptor binding assays was generated. In this fusion protein the AP was located N-terminal of the CXCL14. Conditioned medium from P-CXCL14- or control AP-expressing cells was used as an affinity reagent to stain normal and cancerous mammary tissue sections. Blue staining indicated the presence of a CXCL14 binding protein in certain leukocytes and breast epithelial cells. These findings suggest the presence of a cell surface CXCL14 binding protein(s) in cancerous and normal mammary epithelial cells and are consistent with a paracrine mechanism of CXCL14 action in the breast. To test further the binding characteristics of AP-CXCL14, *in vitro* ligand binding assays were

carried out using various cell lines. Low level AP-CXCL14 binding was detected in all cell lines tested including MDA-MB-231 and MDA-MB-435 breast cancer and MCF10A immortalized mammary epithelial cells (data not shown). To further characterize the AP-CXCL14-putative CXCL14 receptor interaction, more detailed binding assays were carried out on MDA-MB-231 breast cancer cells. Scatchard plot analysis showed two binding slopes in MDA-MB-231 cells, thereby indicating the presence of high ($Kd=6.1x10^{-8}$ M) and low affinity ($Kd=56.7x10^{-8}$ M) binding sites (Fig. 6A).

In previous studies, CXCL12 was demonstrated to enhance breast cancer cell growth, migration and invasion [Hall et al. (2003) Mol. Endocrinol. 17:792-803; Muller et al. (2001)] and it was hypothesized to be involved in metastasis [Kang et al. (2003) Cancer Cell 3:537-549; Muller et al. (2001)]. The present demonstration that it is highly expressed in myofibroblasts from DCIS, a pre-invasive tumor, indicates that it is likely to have additional roles in earlier stages of breast tumorigenesis. In order to determine if CXCL14 has similar effects, the effect of conditioned medium containing AP-CXCL14 on the growth of MDA-MB-231 and MCF10A cells was tested and its effect on cell migration and invasion was investigated using MDA-MB-231 cells. Conditioned media of cells transfected with AP alone and CXCL12 were used as negative and positive controls, respectively. Similar to CXCL12, AP-CXCL14 enhanced the proliferation of MDA-MB-231 and MCF10A cells and the migration and invasion of MDA-MB-231 cells (Figs. 6B and C and data not shown). In these experiments, the concentration of AP-CXCL14 was 2-30 nM, which is similar to the concentration ranges of several chemokines, including CXCL12, required for biological effects. The same results were obtained in cell migration and invasion assays using CXCL14-AP (C-terminal AP-tag) and CXCL14-HA (C-terminal HA-tag) fusion proteins (Fig. 6C and data not shown). Thus, the observed effects are not likely to be due to the position or identity of the epitope tag. Further suggesting that mammary epithelia cells have a functional CXCL14 receptor, experiments using recombinant CXCL14 protein and CXCL14 expressing adenovirus demonstrated the induction of calcium flux in MDA-MB-231 and activation of Akt kinase in MCF10A cells, respectively (data not shown).

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.